From meadows to milk to mucosa - adaptation of Streptococcus and Lactococcus species to their nutritional environments

Price, Claire E; Zeyniyev, Araz; Kuipers, Oscar P; Kok, Jan

Published in:
FEMS Microbiology Reviews

DOI:
10.1111/j.1574-6976.2011.00323.x

Publication date:
2012

REVIEW ARTICLE

From meadows to milk to mucosa – adaptation of *Streptococcus* and *Lactococcus* species to their nutritional environments

Claire E. Price[1,2,3,4], Araz Zeyniyev[1], Oscar P. Kuipers[1,3,4,5] & Jan Kok[1,5]

[1]Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; [2]Biochemistry Department, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; [3]Kluyver Centre for Genomics of Industrial Fermentation, Delft, The Netherlands; [4]Netherlands Consortium for Systems Biology, Amsterdam, The Netherlands; and [5]Top Institute Food and Nutrition, Wageningen, The Netherlands

Correspondence: Jan Kok, Nijenborgh 7, 9747 AG, The Netherlands. Tel.: +31 50 363 2111; fax: +31 50 363 2348; e-mail: jan.kok@rug.nl

Present address: Araz Zeyniyev, Institute of Medical Microbiology, Virology & Hospital Hygiene, University Hospital Rostock, Schillingallee 70, D–18057, Rostock, Germany

Received 25 August 2011; revised 20 December 2011; accepted 21 December 2011. Final version published online 30 January 2012.

DOI: 10.1111/j.1574-6976.2011.00323.x

Editor: Michael Bott

Keywords
lactic acid bacteria; fermentation; metabolism; comparative genomic analysis; virulence.

Abstract

Lactic acid bacteria (LAB) are indigenous to food-related habitats as well as associated with the mucosal surfaces of animals. The LAB family *Streptococcaceae* consists of the genera *Lactococcus* and *Streptococcus*. Members of the family include the industrially important species *Lactococcus lactis*, which has a long history safe use in the fermentative food industry, and the disease-causing streptococci *Streptococcus pneumoniae* and *Streptococcus pyogenes*. The central metabolic pathways of the *Streptococcaceae* family have been extensively studied because of their relevance in the industrial use of some species, as well as their influence on virulence of others. Recent developments in high-throughput proteomic and DNA-microarray techniques, in *in vivo* NMR studies, and importantly in whole-genome sequencing have resulted in new insights into the metabolism of the *Streptococcaceae* family. The development of cost-effective high-throughput sequencing has resulted in the publication of numerous whole-genome sequences of lactococcal and streptococcal species. Comparative genomic analysis of these closely related but environmentally diverse species provides insight into the evolution of this family of LAB and shows that the relatively small genomes of members of the *Streptococcaceae* family have been largely shaped by the nutritionally rich environments they inhabit.

Introduction

Lactic acid bacteria (LAB) are indigenous to food-related habitats as well as associated with the mucosal surfaces of animals (Teuber, 1995; Wood & Warner, 2003). LAB have long been used in the production of dairy, plant, and meat fermented food products, contributing to the taste and texture of the food products. They prevent spoilage by lowering the pH of the product and/or producing growth-inhibiting bacteriocins (Cotter *et al.*, 2005). The use of LAB in milk fermentation is an ancient process dating back to the Early Neolithic period (Evershed *et al.*, 2008). Many LAB species are also used as probiotics for human and animal consumption (Gilliland, 1989) and have generally regarded as safe (GRAS) Food and Drug Administration (FDA) status. In addition, most LAB are quite acid resistant and certain strains are able to effectively survive passage through the gastrointestinal (GI) tract, making LAB attractive potential delivery vehicles for recombinant protein vaccines (Medina *et al.*, 2010). However, not all LAB species have a positive impact on food production or human health. Some can cause food spoilage, while several important (human) pathogenic species are included in the large clade of the LAB.

As their name suggests, lactic acid is the main end product of sugar fermentation in this group of microorganisms. While there are many bacteria that produce lactic acid, the umbrella-term LAB are generally restricted to the genera *Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* (Holzapfel

& Wood, 1995; Vandamme *et al.*, 1996). The classification of LAB remains an unresolved issue because the historical phenotypic classification does not match the rRNA-based phylogeny (Vandamme *et al.*, 1996). Historically, fermentative patterns were largely used to classify LAB and divide them into their various genera and species. This is particularly problematic for dairy *Lactococcus lactis* strains as they can carry multiple plasmids. The plasmid complement easily exceeds 150 kb, representing an important fraction of the *L. lactis* genome. Lactococcal plasmids specify many important metabolic functions such as lactose metabolism, citrate utilization, and peptide and amino acid uptake (Siezen *et al.*, 2005). It is now, however, generally accepted that bacterial classification should reflect the natural, that is, phylogenetic, relationships between bacteria, and species are more often defined by percentage similarities between the 16S or 23S rRNA sequences (Woese, 1987). Despite the introduction of sequence identity criteria for species definition, the classification of genus and family can still be difficult to define, representing agglomerates of species and genera, respectively.

Most LAB belong to the phylum *Firmicutes*, class *Bacilli*, and order *Lactobacillales*. The newly available whole-genome sequences of various LAB have allowed the construction of phylogenetic trees with increased resolution and robustness (Makarova *et al.*, 2006) (Fig. 1a). Maximum representation with parsimony (MRP) supertrees for LAB derived from 1160 single gene families have been generated (Kelly *et al.*, 2010) and indicate similar relatedness to *rpoA*-based trees. Three closely related lineages can be defined: the *Leuconostoc* group, the *Lactobacillus casei-Pediococcus* group, and the *Lactobacillus delbrueckii* group. Further, the *Pediococcus* group is a sister to the *Leuconostoc* group within the *Lactobacillus* clade. The family *Streptococcaceae*, consisting of the *Lactococcus* and *Streptococcus* genera, forms a separate lineage and will be the focus of this review.

Despite the high sequence similarity between members of the family of *Streptococcaceae*, they inhabit a diverse range of environments. Members of the *Lactococcus* genus can be found in grasslands and are widely used in food fermentation, while the *Streptococcus* genus contains both health-promoting and disease-causing and tooth decay–causing species. While *L. lactis* is the best-known lactococcal species, six additional species have been added to this genus. These are *Lactococcus garvieae*, *Lactococcus piscium*, *Lactococcus plantarum*, *Lactococcus raffinolactis*, *Lactococcus chungangensis*, and *Lactococcus fujiensis*, which have been isolated from diverse habitats ranging from fish to activated sludge foam (Schleifer, 1987; Williams *et al.*, 1990; Cho *et al.*, 2008; Cai *et al.*, 2011). *Lactococcus lactis* is further subdivided into four subspecies: *L. lactis* ssp. *cremoris*, *L. lactis* ssp. *hordniae*, *L. lactis* ssp. *lactis*, and the newly identified *L. lactis* ssp. *tructae* (Perez *et al.*, 2011). Historically, *L. lactis* ssp. were referred to as lactic streptococci but were reassigned to their own genus in 1985 in order to distinguish them from the pathogenic streptococci (Schleifer *et al.*, 1985; Schleifer, 1987). A phylogenetic tree based on 16S rRNA gene alignments of the *Lactococcus* species isolated thus far reiterates discrepancies between 16S rRNA gene sequence information and phenotypic characteristics of *Lactococcus* species (Fig. 1b). For example, the closely related *L. lactis* ssp. *cremoris* and *L. lactis* ssp. *tructae* were isolated from diverse environments – dairy products and intestinal mucous of trout, respectively – and have very different carbohydrate utilization patterns (Perez *et al.*, 2011). The classification of *L. lactis* ssp. *lactis* and ssp. *cremoris* is especially problematic because of the occurrence of 'cremoris type' genotypes with 'lactis type' phenotypes. A good example for this is the type stain *L. lactis* ssp. *cremoris* MG1363. The reason for this is that while the 16S rRNA gene sequences of *L. lactis* ssp. *lactis* and ssp. *cremoris* differ by $< 0.7\%$ (Salama *et al.*, 1991), they share only 85% identity at the genome level (Wegmann *et al.*, 2007). This value is only slightly higher than that between *Escherichia coli* and *Salmonella typhimurium* (McClelland *et al.*, 2001).

Like many other LAB, *Streptococcus thermophilus* is used in dairy starter cultures but the *Streptococcus* genus also contains members that are pathogenic to humans and other mammals. *Streptococcus mutans* and *Streptococcus pneumoniae* have been particularly well studied owing to their impact on human health. *Streptococcus mutans* is one of the major causative agents of dental caries (tooth decay) because of its rapid acidification of the environment caused by the end products of its sugar metabolism, while *S. pneumoniae*, also known as pneumococcus, can cause invasive diseases such as pneumonia and meningitis (Musher, 1992; Mufson, 1999; Mason *et al.*, 2007).

The central metabolic pathways of the *Streptococcaceae* family have been extensively studied because of their relevance in the industrial use of some species (e.g. *L. lactis*), as well as their influence on virulence (e.g. *S. pneumoniae* and *Streptococcus pyogenes*). Recent developments in high-throughput proteomic and DNA-microarray techniques, in *in vivo* NMR studies, and importantly in whole-genome sequencing have resulted in new insights into the metabolism of the *Streptococcaceae* family. In this review, we will discuss the central metabolic pathways and their regulation, including the heme-dependent aerobic electron transport chain (ETC) and the ATP-generating arginine deiminase (ADI) pathway, present in the *Streptococcaceae*. Special emphasis will be on the adaptation of metabolic pathways of the sequenced strains to the nutrient-rich environments they inhabit.

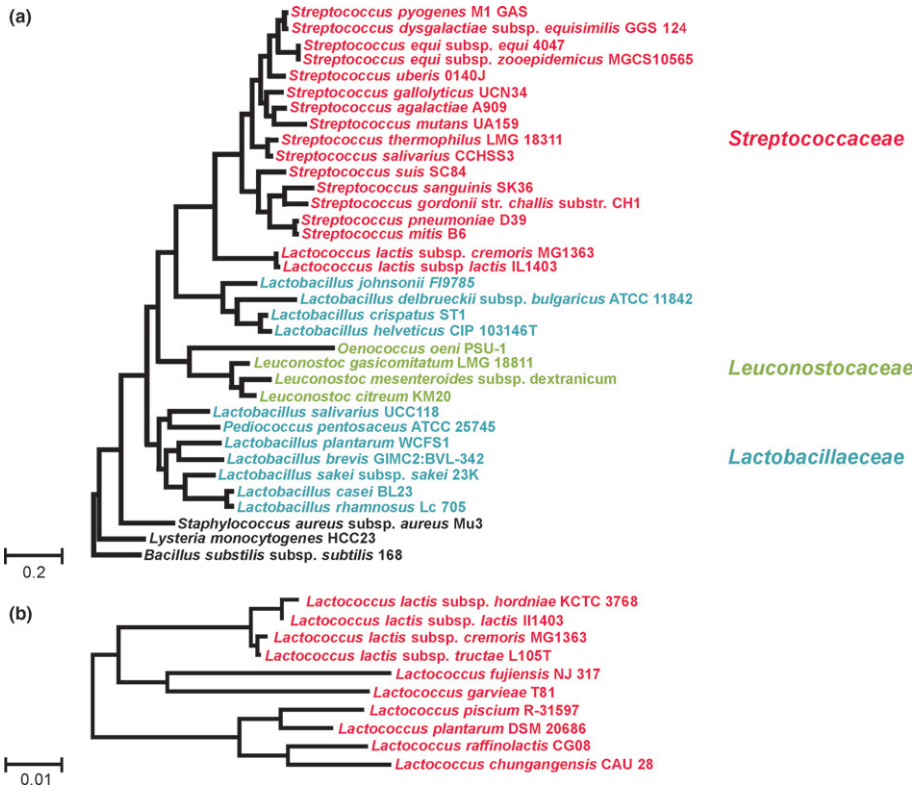

Fig. 1. Phylogenetic trees of LAB and *Lactococcus* species. (a) Rooted phylogenetic tree of sequenced LAB constructed on the basis of alignments of the DNA-dependent RNA polymerase. (b) Phylogenetic tree of *Lactococcus* species on the basis of complete 16S rRNA gene sequences. The minimum-evolution trees were built using MEGA 5.0 software. The species are colored according to the current taxonomy: *Lactobacillaceae*, blue; *Leuconostocaceae*, green; *Streptococcaceae*, red. Outgroup species are indicated in black. Based on Makarova *et al.* (2006).

Central metabolism

Metabolic energy can be defined as the free energy stored in the ATP pool and in the electrochemical protein gradient across the cytoplasmic membrane. Lactococci and streptococci are classified as facultative anaerobes, unable to fully oxidize sugars to CO_2 via the TCA cycle, even when oxygen is present. Under anaerobic conditions, there is no net production of reducing equivalents and ATP is generated by substrate-level phosphorylation. These bacteria convert a large fraction of the hexose sugar substrate to pyruvate via glycolysis and then to lactate in the redox balance step. Alternatively, pyruvate is converted to the mixed-acid products formate, ethanol, acetate, 2, 3-butanediol, and acetoin, generating extra ATP (Kandler, 1983). In a detailed study on *L. lactis* ssp. *lactis* NCDO 2118 using ^{14}C-labeled glucose and amino acids, it was observed that 91% of the fermentation end products were derived from glucose, while the other 9% were derived from serine via pyruvate (Novak & Loubiere, 2000). Relative to the carbon flux from hexose sugars to lactate, the calculated carbon flux to biomass through the pathways branching from glycolysis was small: 5% with growth on minimal medium (Novak & Loubiere, 2000). In addition to glucose, glutamate-derived products formed a large fraction of the biomass. The study reiterated the current notion that glucose is only a catabolic substrate.

Sugar import

Most hexose sugars and disaccharides used by lactococci and streptococci are taken up by phosphotransferase systems (PTSs) but non-PTS permeases are also present (Fig. 2). For example, in *L. lactis* glucose can be imported via the mannose or cellobiose PTSs or the glucose permease GlcU (Castro *et al.*, 2009). In *S. mutans* alone, PTS systems have been identified for glucose (Schachtele & Mayo, 1973), mannitol, glucitol (Brown & Wittenberger, 1973; Maryanski & Wittenberger, 1975), lactose (Calmes, 1978), sucrose (Slee & Tanzer, 1979; St Martin & Wittenberger, 1979), mannose (Vadeboncoeur, 1984; Néron &

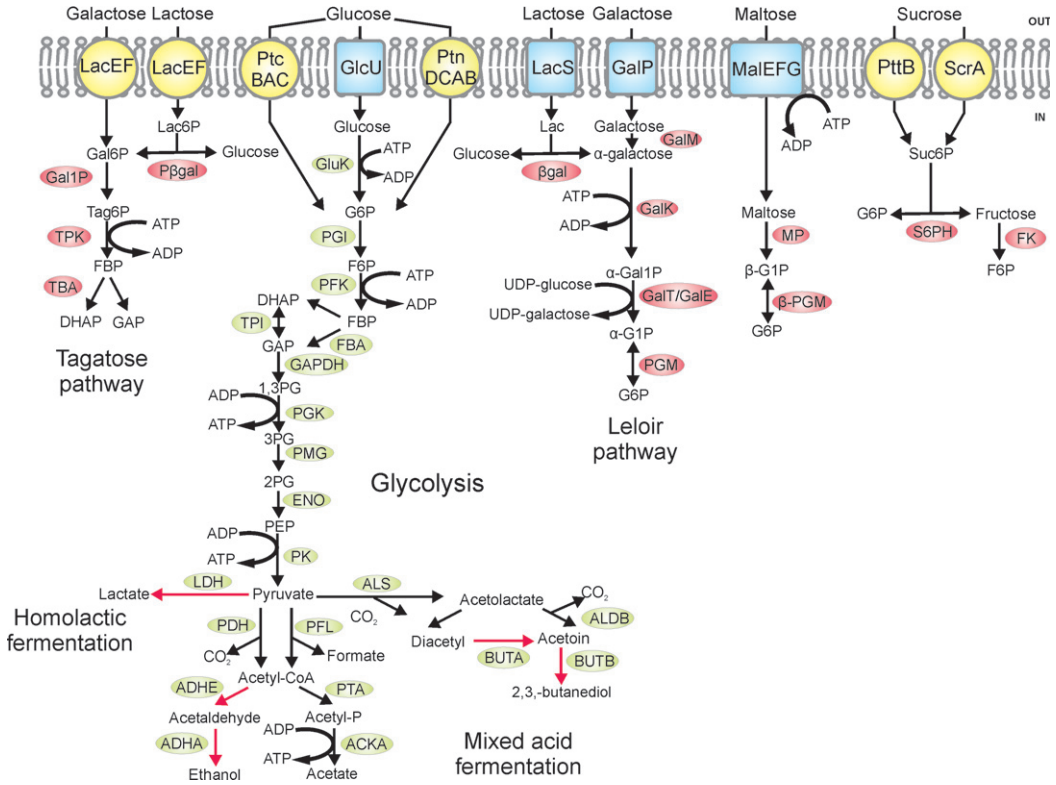

Fig. 2. Hexose and disaccharide sugar utilization in the family of *Streptococcaceae*. Glucose is either internalized and concomitantly phosphorylated the PTS systems (PtcABC or PtnABCD) or imported by the GlcU permease and phosphorylated by glucose kinase. PTS systems are represented by circles, while non-PTS sugar import systems are represented by squares. Glucose-6-phosphate (G6P) is then converted to fructose-6-phosphate (F6P) by phosphoglucose isomerase (PGI) and subsequently to fructose-1,6-bisphosphate (FBP) by phosphofructokinase (PFK). FBP is converted by fructose-bisphosphate aldolase (FBA) to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (GAP), two products that can be interconverted by triosephosphate isomerase (TPI). GAP is in turn converted to pyruvate via 1,3PG, 3PG, 2PG, and phosphoenolpyruvate (PEP), reactions that are catalyzed by GAP dehydrogenase (GAPDH), phosphoglycerate kinase (PGK), phosphoglycerate mutase (PMG), enolase (ENO), and pyruvate kinase (PK), respectively. PEP can also be converted to pyruvate via the sugar PTSs. In the homolactic fermentation pathway, pyruvate is converted to lactate by lactate dehydrogenase (LDH), which uses the reducing equivalents generated in glycolysis. In the mixed-acid fermentation pathway, pyruvate is converted to acetyl-CoA by pyruvate formate lyase (PFL) (under anaerobic conditions) producing formate or by pyruvate dehydrogenase (PDH) (under aerobic conditions). Acetyl-CoA is subsequently converted to ethanol and acetate by acetaldehyde-CoA/alcohol dehydrogenase (ADHE) and alcohol dehydrogenase (ADHA), and phosphotransacetylase (PTA) and acetate kinase (ACKA), respectively. The formation of acetate from acetyl-P generates ATP. When pyruvate levels are high, acetoin and 2,3-butanediol are formed (Snoep *et al.*, 1992) through reactions that are catalyzed by acetolactate synthase (ALS), alpha-acetolactate decarboxylase (ALDB), acetoin reductase (BUTA), and 2,3-butanediol dehydrogenase (BUTB). When galactose or lactose is utilized, each of these sugars is fed into glycolysis by either the tagatose or Leloir pathways. In the tagatose pathway, lactose is imported by the lactose PTS system (LacEF) and the lactose-6-phosphate (Lac6P) formed is hydrolyzed internally by phospho-β-galactosidase (Pβgal) into glucose and galactose-6-phosphate (Gal6P). Galactose is also imported by the lactose PTS system, forming galactose-6-phosphate. The glucose (from lactose) is metabolized to pyruvate via glycolysis, while Gal6P is converted to tagatose-6-phosphate (T6P) by galactose-1-phosphate (Gal1P) and subsequently to FBP by tagatose-phosphate kinase (TPK). FBP is converted by tagatose-bis-phosphate aldolase (TBA) to DHAP and GAP, which can then be fed into glycolysis. All genes involved in the lactose pathway, *lacABCDFEG*, are contained in one operon that is often found on plasmids (de Vos & Vaughan, 1994). In the Leloir pathway, lactose is imported by the lactose permease, LacS, after which it is hydrolyzed into glucose and α-galactose by converted β-galactosidase. Galactose is taken up by the galactose permease (GalP) and converted to α-galactose by galactokinase (GalM). The α-galactose is phosphorylated by galactokinase (GalK) to form α-galactose-1-phosphate (α-Gal1P) from which α-glucose-1-phosphate is formed by galactose-1-phosphate uridylyltransferase/UDP-glucose 4-epimerase (GalT/GalE). From this, glucose-6-phosphate can then be formed by phosphoglucomutase (PGM). Sucrose is imported via the sucrose-specific PTS, ScrA, or via PttB, a trehalose-specific PTS with low affinity for sucrose. Sucrose can also be imported by MSM (multiple sugar metabolism) systems, which are ABC-type transporters in *Streptococcus* species (Russell *et al.*, 1992) (not illustrated). PTS-imported and phosphorylated sucrose-6-phosphate (Suc6P) is hydrolyzed by sucrose-6-phosphate hydrolase (S6PH) to G6P and fructose. The fructose is subsequently phosphorylated by fructokinase. Maltose is also imported by an ABC-type transporter, MalEFG, and maltose is degraded to β-glucose-1-phosphate (β-G1P) by maltose phosphorylase (MP), which is converted in a reversible reaction to G6P by β-phosphoglucomutase (β-PGM). The highlighted arrows indicate reactions in which the NAD^+ needed for ATP synthesis during glycolysis is regenerated. Nomenclature used is for *Lactococcus lactis*.

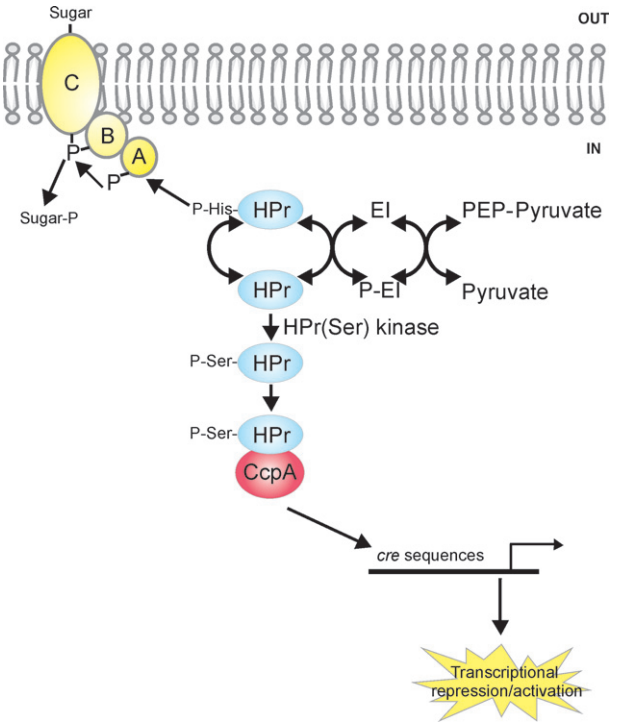

Fig. 3. Hpr- and CcpA-mediated catabolite repression. During carbohydrate uptake and phosphorylation, HPr becomes phosphorylated at His-15 by PEP and enzyme I of the PTS. HPr-His-P acts as a phosphocarrier transferring its phosphoryl group to a sugar-specific EIIA. The phosphoryl group is in turn donated to the corresponding EIIB, after which it is finally transferred to the carbohydrate bound to EIIC. When HPr is phosphorylated at Ser-46 by HPr kinase/HPr-Ser-P phosphatase, it binds to the transcriptional regulator CcpA. CcpA complexed with the HPr-Ser-P binds to certain *cre* sites ensuring hierarchal and efficient use of preferred sugars.

Vadeboncoeur, 1987), fructose (Gauthier *et al.*, 1984a, b), maltose (Würsch & Koellreutter, 1985), and trehalose (Poy & Jacobson, 1990).

When phosphorylated at His-15 by the PEP and enzyme I, the HPr protein acts as a phosphocarrier for sugar PTSs (Fig. 3). HPr-His-P can also function in the regulation of carbohydrate metabolism. HPr-His-P can phosphorylate histidyl resides in non-PTS proteins such as glycerol kinase (Charrier *et al.*, 1997) and non-PTS transporters that contain an $EIIA^{Glc}$ domain (Gunnewijk *et al.*, 1999), stimulating their activity. HPr can be phosphorylated at Ser-46 by the metabolite-controlled HPr kinase/P-Ser-HPr phosphatase. HPr-Ser-P protein plays a role in catabolite repression (Deutscher *et al.*, 1995; Luesink *et al.*, 1999). HPr-Ser-P has also been implicated in inducer exclusion (Saier, 1989; Saier *et al.*, 1996; Titgemeyer & Hillen, 2002), and for *Streptococcus salivarius*, it has been shown that sugar uptake is relieved from glucose inhibition in HPr mutants or mutants unable to form HPr-Ser-P and glucose is no longer preferentially taken up and metabolized over lactose in the mutants (Gauthier *et al.*, 1997; Monedero *et al.*, 2001a, b). This mode of regulation is not observed in *S. mutans* but is present in other streptococci such as *S. pyogenes* (Reizer & Panos, 1980; Reizer *et al.*, 1983). It was previously thought that HPr-Ser-P was directly involved in inducer expulsion. In inducer expulsion, the cell rapidly ejects the nonpreferred carbon sources when exposed to easily metabolized ones, most often glucose. This applies to sugars internalized by PTS. The accumulated sugar-P is dephosphorylated by a sugar-P phosphohydrolase and then expelled into the external medium (Thompson & Saier, 1981; Reizer *et al.*, 1983; Reizer & Saier, 1983; Viana *et al.*, 2000). Recent experiments, however, have shown that HPr-Ser-P does not function in inducer expulsion in *L. lactis* and *Lactobacillus casei* as mutants unable to form HPr-Ser-P expelled nonmetabolizable methyl β-D-thiogalactoside (TMG) (Monedero *et al.*, 2001a, b; Dossonnet *et al.*, 2000).

In addition, HPr acts as an allosteric effector to regulate transcription through the catabolite control protein CcpA. CcpA is a transcriptional regulator that binds to *cis*-elements designated *cre* sites (catabolite response elements) and can act as both an activator and a repressor of gene expression (Luesink *et al.*, 1998). CcpA binds certain *cre* sites, such as those upstream of catabolic operons involved in galactose and maltose utilization, together with the serine-phosphorylated form of HPr, thus ensuring hierarchal and efficient use of preferred sugars (Titgemeyer & Hillen, 2002; de Vos & Vaughan, 1994; Luesink *et al.*, 1998; Deutscher *et al.*, 1995) (Fig. 3). In *L. lactis* ssp. *cremoris*, the link between either gene repression or activation by CcpA and the location of the *cre* site has been investigated (Zomer *et al.*, 2007). Gene activation was observed when *cre* sites were located upstream of the −35 sequence, while repression was observed when the *cre* site was in or downstream of the putative −35 and −10 sequences.

Glycolysis

During glycolysis, ATP is generated, a reaction that requires NAD^+. Glucose imported by PTSs is concomitantly phosphorylated and fed into glycolysis. Other hexose sugars or disaccharides must first be converted and/or hydrolyzed to sugar phosphate species that can be glycolysed (Fig. 2). The conversion of lactose and sucrose into glycolytic intermediates has been extensively studied in *L. lactis* and *S. mutans*, respectively. *Streptococcus mutans* is the major cause of dental caries owing to its formation of the weak acid lactate from ingested sucrose (Munro *et al.*, 1991). Although this bacterium can hydrolyze sucrose extracellularly by secreting fructosyl- and glucosyltransferases (Newbrun, 1983), the majority of dietary

sucrose is metabolized intracellularly (Tanzer *et al.*, 1972). Sucrose is imported and phosphorylated via either the dedicated sucrose PTS or the trehalose PTS (Poy & Jacobson, 1990) after which phosphorylated sucrose is hydrolyzed to glucose-6-phosphate (G6P) and fructose, the latter of which is subsequently phosphorylated by fructokinase to fructose-6-phosphate (F6P) (Postma *et al.*, 1993; Chassy & Porter, 1979). The sugar phosphate compounds can then be channeled into the glycolytic pathway. Galactose and lactose are converted to glycolytic intermediates in *L. lactis* ssp. via either the tagatose or Leloir pathways (summarized in Fig. 2). The main difference between the two pathways is the manner in which the sugars are imported. In the tagatose pathway, lactose and galactose are imported and concomitantly phosphorylated via the PTS^{lac} (Van Rooijen *et al.*, 1991), while in the Leloir pathway the sugars are imported by dedicated permeases (Grossiord *et al.*, 2003; Thomas *et al.*, 1980; Thompson, 1980). This influences the products formed, with the tagatose pathway forming G6P and the trioses, DHAP and GAP, and the Leloir pathway producing G6P and glucose. The use of the Leloir and/or the tagatose pathway for galactose utilization is strain dependent (Thomas *et al.*, 1980).

Control of glycolytic flux

No single enzymatic step controls the rate of glycolysis in *L. lactis*, and control appears to be distributed over many glycolytic steps, sugar uptake, and lactate efflux (Koebmann *et al.*, 2002). This is supported by experiments that modulated glyceraldehyde-3-phosphate dehydrogenase (GAPDH), L-lactate dehydrogenase (LDH), phosphofructokinase (PFK), or pyruvate kinase (PK) expression individually. Decreasing but not increasing GAPDH expression had an effect on the glycolytic flux (Solem *et al.*, 2003), while if the expression levels of LDH, PFK, and PK were increased or decreased together, the flux through glycolysis decreased (Koebmann *et al.*, 2002). Another study showed that decreasing PFK levels alone in *L. lactis* did reduce the glycolytic flux and growth rate (Andersen *et al.*, 2001). The reason for this discrepancy may be that different strains and/or methods were used.

The distribution of flux control over a number of enzymatic steps is partly regulated by CcpA. In addition to controlling the transcription of sugar-specific operons, CcpA acts as an activator of transcription of the *las* operon genes *pfk*, *pyk*, and *ldh*, encoding PFK, PK, and LDH, by binding to a *cre* site upstream of the operon (Luesink *et al.*, 1998; Karlin *et al.*, 2004). CcpA and the metabolite fructose-1, 6-bisphosphate (FBP) mediate both transcriptional and allosteric activation of LDH to ensure the rapid formation of lactate. In *Bacillus subtilis*, FBP has been shown to enhance the interaction of HPr-Ser-P with CcpA and the interaction of the complex with *cre* sites (Seidel *et al.*, 2005; Schumacher *et al.*, 2007). In addition, FBP acts as allosteric regulator of LDH, increasing its activity (Hillier & Jago, 1982). High levels of FBP result in the activation of *pyk* by CcpA in turn decreasing the amount of PEP, thus fine-tuning PTS sugar transport. Regulation by intracellular FBP levels applies to high glycolytic fluxes. When the glycolytic flux is low, the flux is controlled by the ATP demands of the cell (Koebmann *et al.*, 2002).

Homolactic and mixed-acid fermentation

The pyruvate generated by glycolysis can have a number of fates in streptococci and lactococci. In general, under anaerobic conditions and when easily utilizable sugars, that is, glucose, are in excess, pyruvate is converted solely to lactate by LDH. Alternatively, pyruvate can be channeled through the pyruvate formate lyase (PFL) pathway forming formate, ethanol, and acetate. Under aerobic conditions, PFL is inhibited and is replaced by pyruvate dehydrogenase (PDH) in lactococcal and some streptococcal species or pyruvate oxidase (PO) in other streptococci (Taniai *et al.*, 2008; Melchiorsen *et al.*, 2000). The oxygen sensitivity of these enzymes is important in dental health as they allow streptococci such as *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus sanguis* to compete successfully with other bacteria at sites of the oral cavity that are freely exposed to saliva, while *S. mutans* is restricted to anaerobic sites such as those in between teeth (Abbe *et al.*, 1991). The mixed-acid fermentation pathway in *L. lactis* has an important influence on the flavor of dairy products as the short-chain compounds diacetyl, acetoin, acetate, acetaldehyde, and ethanol all have an effect on the palatability (Cogan & Hill, 1993; Escamilla-Hurtado *et al.*, 1996; Henriksen & Nilsson, 2001; Syu, 2001). Diacetyl and acetoin in particular are major flavor compounds in cheeses with creamy buttery aromas such as cheddar and gouda (Dacremont & Vickers, 1994; Christensen & Reineccius, 1995; Curioni & Bosset, 2002). In contrast to other members of the *Streptococcaceae* family, *S. thermophilus* only utilizes the homolactic fermentation pathway owing to the presence of pseudogenes in some primary metabolic pathways (Pastink *et al.*, 2009).

Switch from homolactic to mixed-acid fermentation

It has been proposed that a rapid flux through glycolysis is likely to result in homolactic fermentation, while diminished rates of sugar metabolism provoke a shift

toward mixed-acid fermentation. For example, when the glycolytic flux is low because of inefficient sugar uptake or lack of tagatose pathway activity, some benefit can be derived from switching to mixed-acid fermentation as it generates more ATP than the homolactic pathway, even though the end products of the mixed-acid pathway are more inhibitory than lactate to the bacterium itself (Loubiere *et al.*, 1997). In *L. lactis* ssp. *lactis* strain IL1403, the rapid uptake and concomitant phosphorylation of fructose, mannose, glucose, or sucrose by efficient PTS systems supports rapid rates of sugar metabolism and therefore homolactic fermentation (Bolotin *et al.*, 2001; Cocaign-Bousquet *et al.*, 1996). In contrast, mixed-acid fermentation is observed for growth on sugars such as galactose, gluconate, xylose, maltose, or ribose, which are not imported by a PTS system. In *L. lactis* strains, such as *L. lactis* ssp. *cremoris* NCDO 712, which contain a plasmid encoding a lactose-specific PTS system, lactose fermentation is homolactic (Gasson, 1983).

Expression levels of genes of the enzymes involved in fermentation alone do not explain the shift between homolactic and mixed-acid fermentation. Triose phosphates have a controlling effect on PFL activity, and during glucose fermentation, when triose phosphate pools are high, the activity of this enzyme would be completely inhibited (Thomas *et al.*, 1980; Takahashi *et al.*, 1982; Garrigues *et al.*, 1997). Also, the activities of LDH and GAPDH can be modulated in an opposing but similar manner by the $NADH/NAD^+$ ratio in the cell resulting in a coordinated effect on flux through the glycolytic and fermentation pathways (Garrigues *et al.*, 1997). The activity of LDH is inhibited by increasing concentrations of NAD^+, while the activity of GAPDH is inhibited by increasing concentrations of NADH. Artificial changing of the $NADH/NAD^+$ ratio in *L. lactis* can redirect fermentation from the homolactic to the mixed-acid type with acetoin and diacetyl produced (De Felipe *et al.*, 1998). Whole-genome sequencing has revealed the presence of at least five NADH dehydrogenase/oxidase genes in the genomes of *L. lactis* ssp. *lactis* strains IL1403 and KF147, *L. lactis* ssp. *cremoris* strain SK11 and four in the genome of *L. lactis* ssp. *cremoris* strain MG1363. Each of these may affect the mode of fermentation.

The fermentation mode can be affected by oxygen availability, and in the case of oral streptococci, this has an important effect on the formation of dental caries. In the deep layers of dental plaque, the supply of sugar is limited, the pH is frequently below 7, and these sites are highly anaerobic (Kenney & Ash, 1969). These conditions favor streptococcal PFLs because the intracellular levels of glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), which inhibit PFL, are low and the PFLs are prevented from irreversible inactivation by oxygen. Also, *S. mutans* has a PFL-activating system that operates optimally at pH 6.8. This system includes a PFL-activating enzyme that itself is activated by an electron transport system that transfers an electron from NADH to the PFL-activating enzyme (Takahashi-Abbe *et al.*, 2003). The resulting rapid acid production in the deep layers of dental plaque likely confers the high cariogenic potential of this microorganism. This would be different to the situation in immature thin dental plaque and superficial layers of dental plaque where there would be transient infiltration of oxygen.

The effect of oxygen on metabolism of *Streptococcaceae*

While members of the family *Streptococcaceae* are most often characterized by their fermentative behavior under anaerobic growth conditions, these bacteria are aerotolerant. *Lactococcus lactis* ssp. and *Streptococcus* species are defined as catalase negative but the presence of the flavoproteins NADH oxidase and NADH peroxidase and a manganese-containing superoxide dismutase allows them to grow under aerobic conditions (Hansson & Häggström, 1984; Sanders *et al.*, 1995).

Despite the presence of these enzymes, exposure to oxygen does result in cell damage because of the formation of reactive oxygen species (Anders *et al.*, 1970; Duwat *et al.*, 1995; Miyoshi *et al.*, 2003). The damaging effects of oxygen are, however, markedly reduced when *L. lactis* is grown in the presence of heme (Sijpesteijn, 1970; Mickelson, 1972; Duwat *et al.*, 2001). This is likely due to a shift from homolactic to mixed-acid fermentation, more complete glucose utilization, and energy generation by NADH oxidation via an ETC. (Duwat *et al.*, 2001). The observation that functional cytochromes are synthesized in heme-containing growth medium was made over 40 years ago (Whittenbury, 1964; Bryan-Jones & Whittenbury, 1969; Sijpesteijn, 1970; Ritchey & Seeley, 1974), the genetic basis for which is evident in the whole-genome sequences published thus far. *Lactococcus lactis* strains all contain genes for menaquinone synthesis (*men* operon), the late steps in heme biosynthesis (*hemH*, *hemK*, *hemN*) as well as those for a membrane-bound NADH dehydrogenase (*noxA*, *noxB*) and cytochrome *d* oxidase and its synthesis (*cytABCD*) (Bolotin *et al.*, 2001; Brooijmans *et al.*, 2007; Wegmann *et al.*, 2007) (illustrated in Fig. 4). Furthermore, a clear build-up of a $\Delta\Psi$ has been demonstrated when heme was added to aerated cultures showing that a functional ETC. is indeed formed in *L. lactis* cells (Brooijmans *et al.*, 2007).

The import of heme is likely performed by the ABC-type iron uptake system encoded by the *fhu* operon (Gaudu *et al.*, 2003). CcpA is important in regulating the

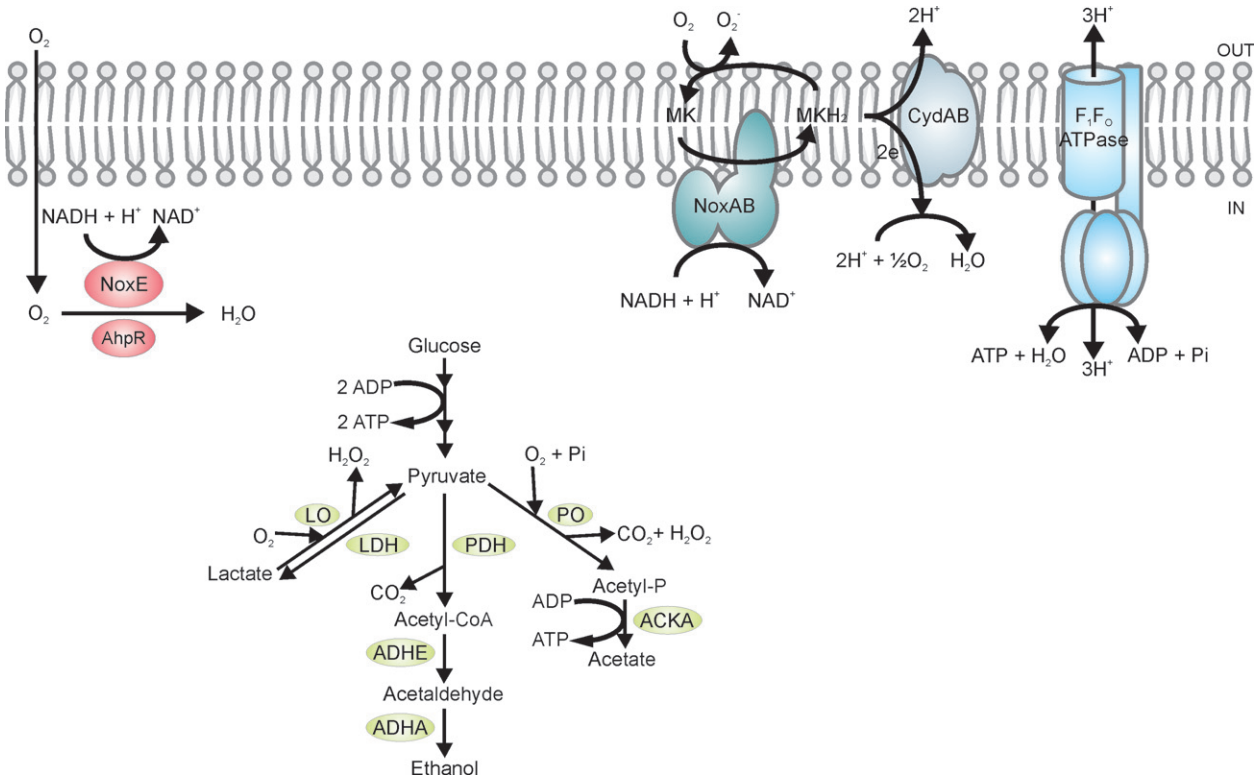

Fig. 4. Oxygen-related metabolism in *Streptococcaceae*. In the absence of heme, *Streptococcaceae* are able to reduce O_2 to H_2O and regenerate NAD^+ via the NADH oxidase, NoxE, together with the alkaline hydroperoxide system (AhpR). Alternatively, in *Lactococcus lactis*, the presence of exogenous heme allows for the synthesis of cytochromes and hence the generation of a PMF via an ETC. The ETC is formed by the membrane-bound NADH dehydrogenase complex (NoxAB) and the cytochrome d oxidase (CydAB). ATP is generated from the PMF built up by the ETC via the F1Fo ATPase. When oxygen is present, pyruvate formate lyase is inactivated and is replaced by pyruvate dehydrogenase (PDH), an enzyme from the mixed-acid pathway. Ethanol is produced from pyruvate via the action of acetaldehyde-CoA/alcohol dehydrogenase (ADHE) and alcohol dehydrogenase (ADHA). Pyruvate can also be converted to acetate via the oxygen-dependent enzyme pyruvate oxidase (PO) and acetate kinase (ACKA). Through the action of lactate oxidase (LO), acetate can be formed from pyruvate, allowing for the production of additional ATP via the mixed-acid pathway.

expression of these genes in the presence of oxygen. Expression of *fhuR*, which encodes a repressor of the heme transport system genes *fhuB*, *fhuG*, and *fhuD*, is activated by CcpA, and in this way, CcpA coordinates oxygen, carbon, and iron metabolism (Gaudu *et al.*, 2003).

The original habitat of *L. lactis*, the plant surface or phyllosphere (Kelly *et al.*, 2010), may contain heme but the actual source is unclear. In the absence of heme, such as is the case in milk, the reduction of O_2 to H_2O is likely due to the NADH oxidase encoded by *noxE* (Jeanson *et al.*, 2009; De Felipe & Hugenholtz, 1999), which is only active in the presence of oxygen. NoxE has been shown to play an important role in the regeneration of NAD^+ during aerobic metabolism in *L. lactis* and *S. mutans* (Higuchi *et al.*, 1993; De Felipe *et al.*, 1997). These bacteria are also able to reduce O_2 to H_2O via the alkyl hydroperoxide reductase (AhpR) composed of the H_2O_2-producing NADH oxidase AhpF and a peroxyredoxine (AhpC), which reduces H_2O_2 to H_2O (Higuchi *et al.*, 1999; Tachon *et al.*, 2010). Genes encoding for thiol peroxidase (*tpx*) and glutathione reductase (*gshR*) have been identified in *L. lactis* and may also be involved in responses of this bacterium to aerobic growth conditions. Notably CcpA is thought to play a role in regulating *noxE* expression in response to oxygen levels (Jensen *et al.*, 2001). Increased NADH oxidase activity would eliminate intracellular oxygen and decrease the NADH pool, which may influence the shift toward mixed-acid fermentation observed in aerobically grown cells (Gaudu *et al.*, 2003).

Streptococcus agalactiae, also known as group B Streptococcus (GBS), is able to respire when heme and menaquinones are supplied (Yamamoto *et al.*, 2005). Respiration is an important virulence factor in *S. agalactiae*, allowing persistence in the blood stream and therefore dissemination of the streptococcal infection (Yamamoto *et al.*, 2005). The heme may be acquired from degraded red blood cells, while the source of menaquinones may be other bacteria present in the host. Respiring *L. lactis* has been shown to provide

S. agalactiae with the menaquinones necessary for respiration (Rezaïki *et al.*, 2008). Not all streptococci are capable of respiration. For example, *S. mutans* lacks cytochromes, heme-containing proteins, and catalases but is able to grow in the presence of oxygen, and all genes known to be involved in oxidative stress are present (Yamamoto *et al.*, 2000; Ajdic *et al.*, 2002).

Gene regulation in response to oxygen plays an important role in the virulence of many species of *Streptococcus*, and the production of H_2O_2 gives them a competitive advantage in their specific niches. This phenomenon has been well studied in *S. pneumoniae* that contains, in addition to the PFL and PK enzymes, the H_2O_2-forming flavoprotein PO. When oxygen is present, pyruvate can be converted to acetate via acetyl phosphate, yielding ATP (Spellerberg *et al.*, 1996) (Fig. 4). Acetyl phosphate is formed by PO, accounting for most of the H_2O_2 produced by aerobically growing *S. pneumoniae* cells (Spellerberg *et al.*, 1996). The production of H_2O_2 has an inhibitory effect on other inhabitants of the upper respiratory tract, such as *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Neisseria meningitidis* (Pericone *et al.*, 2000), and contributes to the virulence of *S. pneumoniae* (Regev-Yochay *et al.*, 2007). The extra ATP generated by this pathway prolongs the survival of *S. pneumoniae* cells exposed to exogenously added H_2O_2 (Pericone *et al.*, 2003). Although H_2O_2 production is lethal to *S. pneumoniae*, causing the stationary-phase apoptosis observed during aerobic growth, it confers a colonization advantage to this bacterium in the upper respiratory tract. This would explain the persistence of such apparently suicidal genes (Regev-Yochay *et al.*, 2007). A novel gene, *poxL*, encoding PO has also been identified in *L. lactis* strains IL1403, MG1363, and KF147 (Bolotin *et al.*, 2001; Wegmann *et al.*, 2007; Siezen *et al.*, 2010). Although this enzyme has not been studied in *L. lactis*, PO activity has been detected under aerobic growth conditions in the LAB *Lb. plantarum* (Goffin *et al.*, 2006).

Streptococcus pneumoniae possesses another H_2O_2-forming flavoprotein, L-lactate oxidase (LO). This enzyme was first described in *S. pneumoniae* in 1959 (Udaka *et al.*, 1959) and catalyzes the formation of pyruvate and H_2O_2 from lactate and O_2. LO is also found in *S. pyogenes* (Seki *et al.*, 2004). The two H_2O_2-forming flavoproteins can act in a concerted manner under aerobic conditions to obtain a greater amount of energy from glucose than under anaerobic fermentative conditions. H_2O_2 formation in *S. pneumoniae* is independent of the glucose concentration, while in *S. pyogenes* there is no H_2O_2 build-up or LO activity until the glucose in the medium is exhausted (Gibson *et al.*, 2000). This is also true for the fish pathogen *Streptococcus iniae* (Gibello *et al.*, 1999). The constitutive expression of the *spx* (PO) and *lox* (LO) genes in *S. pneumoniae* may therefore be unique to this bacterium (Taniai *et al.*, 2008). Still unclear, however, is the achievement of redox balance in the pathways just described. The recycling of NADH is apparently incomplete because only part of the pyruvate derived from glucose is available for NADH oxidation by LDH. It has been suggested that cycling between pyruvate and lactate occurs, in which a portion of the pyruvate pool produced by LO is reduced back to lactate by LDH with concomitant oxidation of NADH. Alternatively, an NADH oxidase could perform the necessary regeneration of NAD^+ (Auzat *et al.*, 1999).

Proton motive force generation by the F1Fo ATPase and solute transport

While many anaerobic bacteria utilize sugars in the ways described above, lactococci and streptococci are unique in their avoidance of *de novo* synthesis of many macromolecular building blocks, especially amino acids (Juillard *et al.*, 1995; Poolman & Konings, 1988). These bacteria therefore make great nutritional demands on the growth medium, which are accommodated by a wide variety of substrate transporters (Poolman, 1993).

Nutrient uptake is an energy-expensive process. Although *L. lactis* has a relatively small genome, analysis of the sequenced genomes reveals that between 8.5% and 10% of the predicted proteins are transporter proteins. This is comparable to *E. coli*, although *L. lactis* strains have a higher proportion of ABC transporters relative to secondary transporters (Konings, 2002). Although energetically costly, ABC transporters usually have very high affinities for their solutes and catalyze transport at high rates. This may allow *L. lactis* to efficiently scavenge essential solutes from the environment, giving it a competitive advantage (Konings, 2002). Transport via secondary transporters, that is, those coupled to the influx of protons driven by the proton motive force (PMF), is less energetically expensive than uptake via ABC transporters. The main proton pump in *L. lactis* and *Streptococcus* species responsible for PMF generation is the F1Fo ATPase, which pumps protons at the expense of metabolic ATP (Maloney, 1982). As already mentioned, under aerobic conditions with a heme source, the ETC. also leads to the generation of a PMF (Brooijmans *et al.*, 2007).

A more energy-efficient manner of nutrient uptake than the ones described above is by symport or antiport in which the passage of compounds across the membrane is coupled resulting in the export of one proton. In *S. mutans*, malate fermentation can be coupled to ATP synthesis (Sheng & Marquis, 2007). In this processes, known as malolactic fermentation, dicarboxylic L-malate is converted to monocarboxylic L-lactate and carbon dioxide. The uptake of malate is coupled to the export of

lactate, resulting in the transport of one proton. The PMF built up in this way can subsequently be used for ATP synthesis by F1Fo ATPase (Poolman *et al.*, 1991). Antiport can also make the cost of transport neutral as, for example, in the case of the arginine/citrulline transporter. Thus, ATP can be generated via the arginine deiminase pathway (discussed in detail below) rather than being used up for arginine transport into the cell.

Although lactate is the main fermentation product of LAB, the manner in which it leaves the cell has not been elucidated for all LAB species. Lactate is a weak acid with a pK_a of 3.87, and at an intracellular pH of 7, almost all of it is dissociated and therefore membrane-impermeable. Biochemical studies in *L. lactis* have provided evidence for lactate symport with two or three protons (Michels *et al.*, 1979; Otto *et al.*, 1980a, b; ten Brink *et al.*, 1985), although the protein(s) responsible for this transport have yet to be identified. The transport of lactate together with protons could therefore generate 0.66–0.5 mol ATP per mol lactate transported. If the continuous production of large quantities of metabolic end products was coupled to protons, it would contribute significantly to the PMF and overall production and conservation of energy (Michels *et al.*, 1979; ten Brink & Konings, 1982). This prediction is supported by the observation that *L. lactis* has an increased biomass yield when co-cultured with *Pseudomonas stutzeri*, which removes the lactate from the medium (Otto *et al.*, 1980a, b).

More important than for the uptake of nutrients, proton translocation is used in the maintenance of a proper internal pH in streptococcal and lactococcal species (Harold, 1986). The internal pH is not fully maintained in these bacteria, and acidification of the cytoplasm does occur when the pH of the external medium decreases. *In vivo* NMR studies in *L. lactis* to measure the internal pH revealed that while the cytoplasmic pH is 7.2 at an external pH of 6.5, it drops to 6 when the external pH is 4.8 (Neves *et al.*, 2002a, b). While the F1Fo ATPase can generate ATP under physiological conditions (Maloney, 1982), it acts predominantly as a proton pump (Konings *et al.*, 1989) and the acid tolerance of LAB is based primarily on this activity (Quivey *et al.*, 2000, 2001; Bender *et al.*, 1986; Suzuki *et al.*, 2000). Ammonium, which is produced during the degradation of arginine, may also function as a neutralizing agent against protons (see ADI pathway below). The enzymes responsible for the production of ammonium are extremely acid tolerant, and in *Streptococcus rattus* and *S. sanguis*, the enzymes have been shown to function at pH values as low as 3 (Casiano-Colón & Marquis, 1988).

ADI pathway

The ADI pathway, through which arginine is degraded into ammonium and carbon dioxide with concomitant production of ATP, is widespread in *Streptococcaceae* (Abdelal, 1979; Cunin *et al.*, 1986). The ADI pathway in *Streptococcus* species is commonly referred to as the arginine deiminase system (ADS) (Quivey *et al.*, 2001).

The ADI pathway consists of the three enzymes, arginine deiminase (ADI), catabolic ornithine carbamoyltransferase (OTC), and carbamate kinase (CK) (Cunin *et al.*, 1986) (Fig. 5a). ADI is encoded by genes designated as either *arcA* (for arginine catabolism) or *sagP* (for streptococcal acid glycoprotein) in some streptococcal species. The enzyme hydrolyzes arginine into citrulline and ammonium. The presence of ADI activity was used historically to differentiate between the *L. lactis* ssp. *lactis* and *cremoris* with *L. lactis* ssp. *lactis* strains all containing active ADI enzymes (Crow & Thomas, 1982). *Lactococcus lactis* ssp. *cremoris* strain MG1363 is atypical in that it possesses a fully functional ADI pathway. The product of ADI, citrulline, is metabolized by catabolic ornithine carbamoyltransferase or ornithine transcarbamoylase (OTC) into ornithine and carbamoyl phosphate. Ornithine, a byproduct, is excreted out of the cell via the arginine–ornithine antiporter (Poolman *et al.*, 1987). Electroneutral exchange of equimolar amounts of arginine and ornithine takes place without the need for an external energy supply. OTC genes are often misannotated; for example *S. mutans arcB* and *L. lactis argF2* are in fact putrescine carbamoyltransferase genes (Naumoff *et al.*, 2004). CK, the product of the *arcC* gene, catalyzes the final step in arginine catabolism, the conversion of carbamoyl phosphate into carbon dioxide and ammonium with concomitant transfer of the high-energy phosphate group to ADP, generating ATP. Carbamoyl phosphate also serves as a substrate for *de novo* pyrimidine biosynthesis. The *arcC* gene is duplicated in many species, either residing in tandem, as in *L. lactis* ssp. *lactis* IL1403 and *L. lactis* ssp. *cremoris* MG1363, or at distant loci on the chromosome as in *L. lactis* ssp. *lactis* KF147.

Besides these genes coding for metabolic enzymes, transcription factor genes such as ArgR, AhrC, and ArcR are also frequently found in the ADI pathway gene cluster, for example, in *L. lactis*, *S. pyogenes*, and *S. pneumoniae*, as is the gene *argS* encoding arginyl-tRNA synthetase (Fig. 5b). The arginine–ornithine antiporter gene *arcD* is also frequently found in the ADI pathway gene cluster. In *L. lactis* MG1363, ArgR functions as a DNA-binding protein repressing the expression of the ADI pathway gene cluster. It has been proposed that ArgR binds DNA in a homohexameric form. AhrC (ArgR homologous regulator C), on the other hand, is an arginine sensor lacking DNA-binding ability. It functions as a cofactor relieving repression by ArgR through the formation of heterohexamers with ArgR (Larsen *et al.*, 2005). The promoter

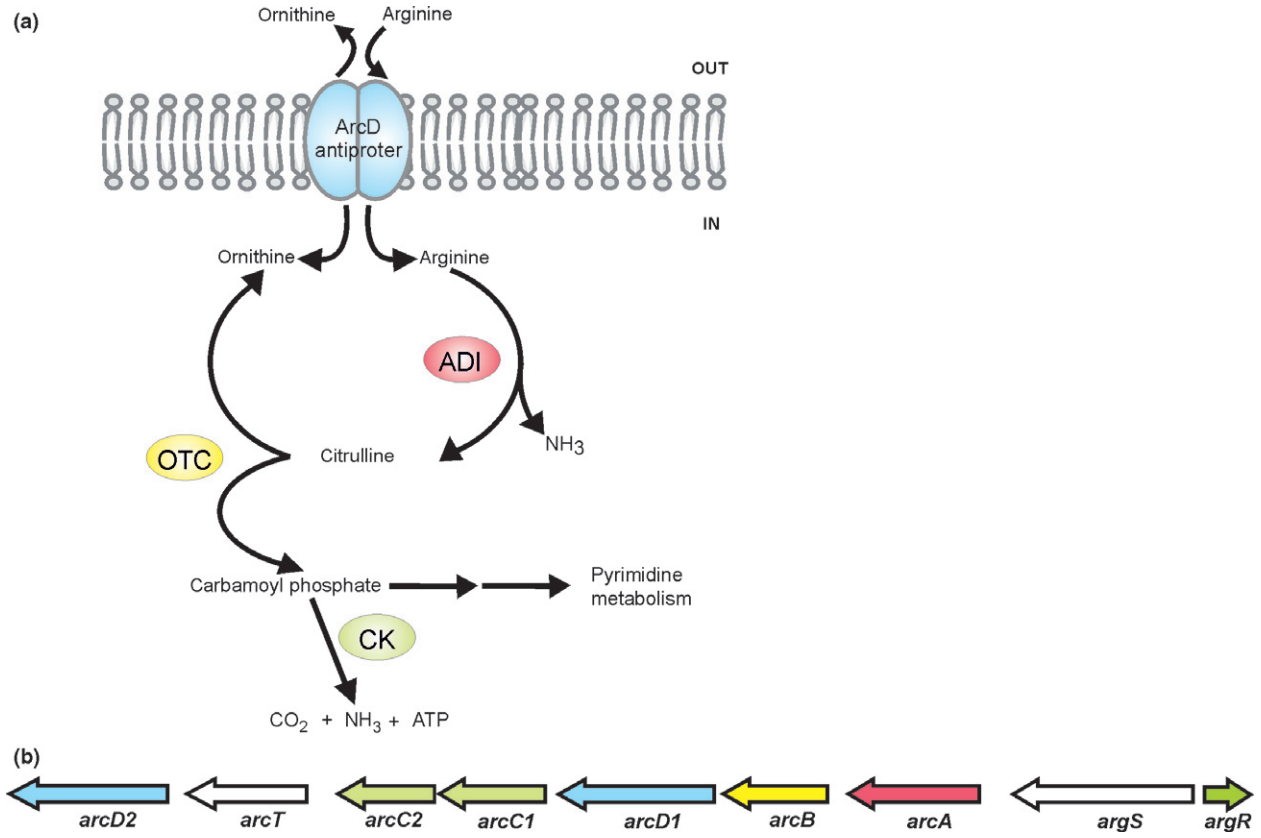

Fig. 5. Arginine deiminase pathway in *Streptococcaceae*. (a) Arginine is imported into the cell via ArcD antiporter in equimolar amounts in exchange for ornithine, which is a waste product of arginine catabolism. In the cytoplasm, arginine is degraded into citrulline and ammonia by the arginine deiminase enzyme (ADI) enzyme. Catabolic ornithine transcarbamoylase (OTC) catalyzes the conversion of citrulline into ornithine and carbamoyl phosphate. Carbamate kinase (CK) then degrades citrulline to carbon dioxide and ammonium with the concomitant production of ATP. Carbamoyl phosphate can also be used as a precursor for de nova synthesis of pyrimidines. (b) Organization of ADI pathway gene cluster in *Lactococcus lactis* IL1403.

regions of the ADI pathway gene cluster of *L. lactis* and *S. gordonii* contain a *cre* site, likely responsible for the repression of these genes by glucose via CcpA (Poolman *et al.*, 1987; Dong *et al.*, 2004). The ADI pathway gene cluster in *S. gordonii* contains two extra genes, namely *flp* and *queA*. The *flp* gene encodes for an Fnr-like protein and has been shown to be a transcriptional activator of the gene cluster (Dong *et al.*, 2004). QueA is predicted to be an *S*-adenosylmethionine:tRNA ribosyltransferase isomerase responsible for the queosine modification of tRNAs. QueA has a role in the modulation of gene expression by exerting a negative effect on the translation of other ADI pathway transcriptional regulators (Liu *et al.*, 2008). Although deletion of either the *queA* or *ccpA* genes had a measurable effect on *arcA* transcription, no changes in ADI enzyme activity were observed (Liu *et al.*, 2008). This suggests that there is tight post-translational control over the amount of ADI pathway enzymes in the cell.

The ADI pathway produces ATP and arginine can support growth in *L. lactis* ssp. *lactis* strains as well as in another LAB, *Enterococcus faecalis* (Niven *et al.*, 1941; Deibel, 1964; Simon *et al.*, 1982). Typical *L. lactis* ssp. *cremoris* strains do not possess the ADI pathway but the effect hereof on pH tolerance and/or ATP production has not been studied.

Evidence from genome sequences for adaptation of *Streptococcaceae* to nutrient-rich environments

The genomes of members of the family *Streptococcaceae* are relatively small, with between 1.8 and 2.5 Mb, and to date (November 2011), complete genome sequences of the two *L. lactis* ssp. *lactis* and *cremoris*, as well as 13 *Streptococcus* species, are available. Examination of lactococcal and streptococcal genomes has provided insight into their phylogenetic relationships and has, importantly,

enabled analysis of the evolutionary paths followed by these bacteria. Analysis of phylogenetic patterns of *Lactobacillales* COGs, together with the phylogenetic tree of this order (Fig. 1), suggests that many of the changes that occurred in *Streptococcaceae* after their divergence from the *Bacillus* ancestor are related to the transition to new nutrient-rich environments such as milk and the GI tract (Makarova *et al.*, 2006; Makarova & Koonin, 2007).

Genome reduction

Striking in the lactococcal and streptococcal genome sequences is the indication of reduced biosynthetic capacities of these bacteria, likely resulting from genome reduction events as they adapted to new nutrient-rich environments. During this process, a number of genes for the biosynthesis of cofactors, such as heme, have been lost, although this is in some cases compensated for by uptake systems. Dairy *L. lactis* strains and particularly those with the 'cremoris' phenotype have smaller chromosomes than wild-type strains (Kelly *et al.*, 2010). Dairy strains have been selected as essential components of industrial processes and have evolved accordingly, largely through the loss of function, so that they are no longer fit to survive outside the dairy environment. Particular to adaptation to dairy environments, many of the amino acid biosynthetic genes in *L. lactis* strains are inactivated or do not produce the amino acids in sufficient amounts to support growth (van Niel & Hahn-Hägerdal, 1999). Most *L. lactis* ssp. *lactis* dairy isolates are auxotrophic for histidine and branched-chain amino acids (BCAA), while most nondairy isolates are prototrophic for histidine and BCAAs. The inactivation of genes for the biosynthesis of these amino acids has therefore been proposed to have occurred during the adaptation to milk (Delorme *et al.*, 1993; Godon *et al.*, 1993). Although the biosynthetic operons for histidine and BCAA are present, mutations such as the nonsense mutations and deletions identified in the *leu* genes in *L. lactis* IL1403 disrupt leucine biosynthesis in this strain (Godon *et al.*, 1993).

The high degree of amino acid auxotrophy observed for *L. lactis* dairy strains is in contrast to the extensive amino acid biosynthetic capability of *S. thermophilus.* This dairy bacterium is only auxotrophic for histidine and methionine or cysteine (Pastink *et al.*, 2009). The operon for histidine biosynthesis is absent, and a truncated *yhcE* gene may contribute to the need for sulfur-containing amino acids (Pastink *et al.*, 2009). In many organisms, TCA enzymes can also have roles in the synthesis of certain amino acid precursors (Fig. 6). This has been observed in *S. mutans* where glutamate and hence glutamine are synthesized from oxaloacetate (Cvitkovitch *et al.*, 1997) (Fig. 6). The *S. pneumoniae* R6 genome, on the other hand, contains none of the eight genes comprising the TCA cycle. *Streptococcus pneumoniae* R6 is therefore incapable of synthesizing aspartate, and therefore lysine, methionine, threonine, and isoleucine from oxaloacetate, nor can it synthesize glutamate, and hence arginine, via α-ketoglutarate.

Adaptation of the family *Streptococcaceae* and LAB in general to oxygen-poor environments is evident from the absence of catalase and cytochrome oxidase–related genes

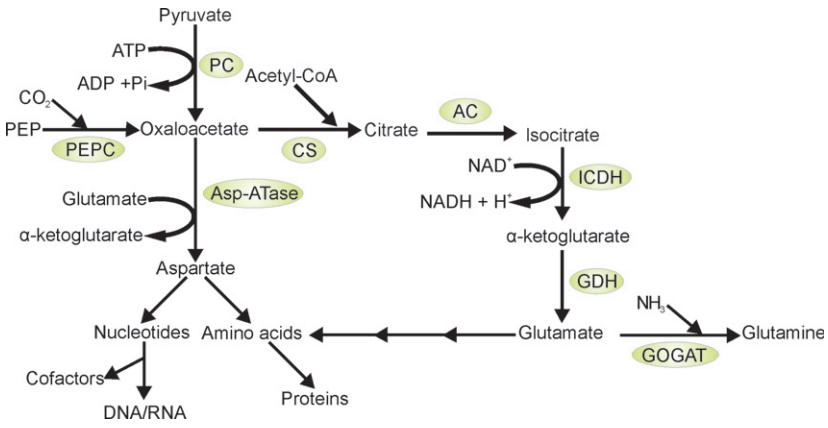

Fig. 6. Proposed oxidative branch of the TCA cycle in *Streptococcaceae*. PEP and pyruvate can be converted to oxaloacetate via PEP (PEPC) and pyruvate carboxylase (PC), respectively. The citrate pathway can be used to synthesize glutamate and hence glutamine. Oxaloacetate and acetyl-CoA are required for the formation of citrate, a reaction catalyzed by citrate synthase (CS). Citrate is subsequently converted to isocitrate by aconitase (AC) and then to α-ketoglutarate by isocitrate dehydrogenase (ICDH). The final step in glutamate synthesis is catalyzed by glutamate dehydrogenase (GDH) after which glutamine can be synthesized via the glutamine synthetase–glutamate synthase pathway (GOGAT). Through the action of aspartate aminotransferase (ASP-ATase), aspartate is formed from oxaloacetate. Aspartate can then be used in biosynthesis reactions to form other amino acids or nucleotides.

(Makarova *et al.*, 2006). Also, no LAB species encodes a complete TCA cycle. The extent of genome reduction varies between the species and is often reflected in the ability of the organisms to synthesize certain amino acids. Further variation in genome reduction is evident within *L. lactis* ssp. where, for example, *L. lactis* ssp. *cremoris* strains MG1363 and SK11 do not contain the *cit* operon for citrate utilization, while *L. lactis* ssp. *lactis* IL1403 does. Citrate utilization was used historically to distinguish between *L. lactis* ssp. but because this phenotype appears to have been unstable and often plasmid-located (Górecki *et al.*, 2011), it was not a reliable marker (Kempler & McKay, 1981).

Genome reduction appears to have been a major factor in the shaping of streptococcal and lactococcal genomes, and all *Streptococcaceae* species show loss of specific genes as well as pseudogenes (Wegmann *et al.*, 2007; Makarova *et al.*, 2006). *Streptococcus thermophilus* in particular has many more pseudogenes than the other *Streptococcus* species sequenced to date. Pseudogenes account for 10% of the 1.8-MB *S. thermophilus* genome, while for other *Streptococcus* species this value is between 0.7% and 4.2% (Bolotin *et al.*, 2004). Notably, the genes encoding four of the seven PTS transporters in *S. thermophilus* are pseudogenes. The adaptation of this bacterium to the dairy environment is further evident from the loss of functionality of many other carbohydrate utilization genes, in line with the limited range of carbohydrates present in milk (Bolotin *et al.*, 2004). Although *S. thermophilus* is phylogenetically close to pathogenic streptococci, most streptococcal virulence–related genes that are not involved in basic cellular functions are absent from or inactivated in the former.

Reductive evolution during the adaptation to dairy environments is evident in the carbohydrate utilization genes contained by the *L. lactis* ssp. sequenced so far. The genomes of *L. lactis* ssp. *lactis* IL1403 (Bolotin *et al.*, 2001) and KF147 (Siezen *et al.*, 2010), as well as those of *L. lactis* ssp. *cremoris* MG1363 (Wegmann *et al.*, 2007) and SK11 (Makarova *et al.*, 2006), have been fully sequenced, representing both plant (KF147) and dairy (the other 3) isolates. The original habitat of *L. lactis* is generally believed to be plants, and this is evident in the ability of plant isolates to degrade plant sugars that the dairy strains cannot. For example, *L. lactis* KF147 can utilize fructans, which are the dominant carbohydrate reserve in many plants, and also contains a gene cluster for the degradation of mannose, polymers of which are present in plant cell walls (Siezen *et al.*, 2008). Some *L. lactis* dairy strains still contain genes or remnants of genes for the utilization of certain plant sugars. For example, carbohydrate utilization experiments with genome sequence information from *L. lactis* MG1363 have shown that this strain can utilize mannitol and sorbitol (Wegmann *et al.*, 2007; Kelly *et al.*, 2010). *Lactococcus lactis* MG1363 as well as *L. lactis* strains IL1403 and SK11 contains cell surface proteins (CscABCD) present in other Gram-positive plant-associated bacteria, supporting the notion of a plant-associated ancestor (Wegmann *et al.*, 2007).

Gene duplication

The adaption of the family *Streptococcaceae* to specific environmental niches is also evident from genetic gains, for example, through the duplication of genes involved in carbohydrate transport and metabolism, including those encoding PTSs, β-galactosidases and L-lactate dehydrogenases and those involved in peptide or amino acid transport and utilization (Makarova & Koonin, 2007). Growth in nutrient-rich environments appears to have been the major driving force behind the fixation of gene duplications during evolution.

Pathogenic streptococcal species contain a particularly large number of sugar uptake systems, specific to the carbohydrates they may encounter. *Streptococcus mutans* is potentially capable of metabolizing a wider variety of carbohydrates than any other Gram-positive organism sequenced to date (Ajdic *et al.*, 2002). Genes with (putative) functions in transport and metabolism of cellobiose, fructose, galactose, glucose, β-glucosides, lactose, maltose/maltodextrin, isomaltosaccharides, mannose, melibiose starch, raffinose, ribulose, sucrose, trehalose, and possibly sorbose are found in the genome of *S. mutans*. The genome of *S. mutans* UA159 may encode as many as 14 PTSs with a variety of enzyme II domains with specificity for different sugars (Ajdic *et al.*, 2002). *Streptococcus mutans* is also able to convert several sugar-alcohols, such as mannitol and sorbitol, to glycolytic intermediates (Ajdic & Pham, 2007). Analysis of the genome sequence of *S. pneumoniae* suggests that this bacterium also has a sugar utilization range wider than other residents of the human upper respiratory tract sequenced to date (*H. influenzae* and *N. meningitidis*) (Tettelin *et al.*, 2000; Taniai *et al.*, 2008). *Streptococcus pneumoniae* is predicted to contain 21 PTS sugar-specific enzyme II complexes with a variety of gene and domain arrangements, which is, relative to genome size, more than twice that in any other sequenced organism (excluding other *Streptococcus* species) and emphasizes the importance of sugars to the lifestyle of *S. pneumoniae*. In addition to the PTSs, *S. pneumoniae* has seven ABC sugar uptake systems, most of which do not have cytoplasmic ATP-binding components encoded with the substrate-binding and membrane components. Like *S. mutans*, *S. pneumoniae* contains genes coding for the catabolism of pentitols via the pentose

phosphate pathway (Tettelin *et al.*, 2001; Ajdic *et al.*, 2002). The ability to use a wide variety of carbohydrates is important for the virulence of pathogenic streptococci, allowing these bacteria to persist and effectively compete in their particular environmental niches (Tettelin *et al.*, 2001; Ajdic *et al.*, 2002; Abranches *et al.*, 2008).

Many *Lactobacillales* species carry two enolase genes, while most bacteria have a single gene for this glycolytic enzyme. Duplicate enolase genes are present in the genomes of *L. lactis* ssp. *lactis* strains IL1403 and KF147 and in *L. lactis* ssp. *cremoris* SK11, while in *L. lactis* ssp. *cremoris* MG1363, *enoB* is a pseudogene (Wegmann *et al.*, 2007). Both enolases in *L. lactis* IL1403 have enzymatic activity (Jamet *et al.*, 2001) but their specific physiological functions remain unclear. In pathogenic bacteria such as *Brucella abortus* (Han *et al.*, 2011) and *Streptococcus suis* (Esgleas *et al.*, 2008), enolase has been shown to be surface located and involved in human–pathogen interactions. The duplicate enolases in some LAB species may therefore have functions separate to glycolysis. Phylogenetic analysis of the duplicate enolase genes of lactobacilli suggests that one of the gene copies is the ancestral version in Gram-positive bacteria, while the other copy was acquired by the *Lactobacillales* ancestor from a different bacterial lineage, likely *Actinobacteria* (Makarova *et al.*, 2006). *Lactococcus lactis* also contains three chromosomally encoded LDH genes – *ldh*, *ldhB*, and *ldhX*. LdhB activity was observed in an *ldh*-deletion strain. The *ldhB* gene, normally silent, could be activated by the insertion of an IS*905*-like element, creating a hybrid active promoter upstream of *ldhB* (Gaspar *et al.*, 2007). Duplications of the enolase and/or lactate dehydrogenase genes are not present in the sequenced genomes of *Streptococcus* species.

The amino acid biosynthetic deficiency in *Streptococcaceae* is offset by scavenging for resources from the environment using many amino acid and peptide uptake systems. The genome of *S. pyogenes* encodes for six ABC transporters putatively identified as amino acid uptake systems, as well as two additional transporter systems that appear to mediate the uptake of dipeptides and oligopeptides (Ferretti *et al.*, 2001). Adaptation to using extracellular proteins or peptides is well documented for *L. lactis* and is discussed below.

Plasmid-borne genes and horizontal gene transfer

As already mentioned, many dairy *L. lactis* strains carry multiple plasmids representing an important fraction of the genome. In addition to the metabolic functions already named, lactococcal plasmids specify protease activity, exopolysaccharide production, bacteriocin production and immunity, and bacteriophage resistance which is especially important for industrial-scale fermentations (Siezen *et al.*, 2005).

Four plasmids from the dairy isolate *L. lactis* ssp. *cremoris* SK11 carry at least nine regions that could have been acquired through horizontal gene transfer (HGT) from enterococci, streptococci, or lactobacilli (Siezen *et al.*, 2005). The plasmid sequences reflect adaptations related to growth on milk components such as lactose and caseins, as well as reveal the presence of genes involved in the uptake of cations such as Mg^{2+}, Mn^{2+}, and possibly Fe^{2+}.

HGT events have also allowed *L. lactis* to inhabit its original plant environment. Genome sequencing of the plant isolate *L. lactis* KF147 revealed the presence of several putative transposons (Siezen *et al.*, 2010). The largest putative transposon contains a gene cluster (*sucP-aga-ABCR-galTK-aga-galR-fbp*) for α-galactoside utilization, the products of which have very high similarity (92% amino acid sequence identity) to those of *L. raffinolactis* ATCC43920 (Boucher *et al.*, 2003). The α-galactosidase enzyme, encoded by *aga*, catalyzes the release of D-galactose moieties from typical plant oligosaccharides such as stachyose, raffinose, and melibiose, making them available for utilization via the Leloir pathway. In both *L. lactis* KF147 and *L. raffinolactis*, this gene cluster has a higher than average G+C content, suggesting that it has been acquired by HGT (Siezen *et al.*, 2008). Conjugative transfer of this transposon from *L. lactis* KF147 to the dairy isolate *L. lactis* NZ4501 has been achieved *in vitro* (Siezen *et al.*, 2011). This genetic element is normally not present in the dairy-derived strains and is spontaneously lost upon prolonged growth of *L. lactis* KF147 in milk (Bachmann, 2009).

Milk contains very low amounts of free amino acids and peptides, and *L. lactis* ssp., having low amino acid biosynthetic capabilities, may depend on a proteolytic system that allows the degradation and utilization of the predominant protein in bovine milk – casein (Mills & Thomas, 1981; Juillard *et al.*, 1995). Casein contains all the amino acids necessary for the growth of *L. lactis*. The proteolytic system consists of an extracellular, cell-wall-anchored proteinase (PrtP) that degrades casein into smaller peptides, transport systems that translocate these peptides into the cell, and peptidases that degrade the peptides intracellularly (Tynkkynen *et al.*, 1993). PrtP degrades casein into more than a hundred different oligopeptides ranging from 4 to 30 amino acid residues, which are then transported into the cell by the OppABCDF ABC transporter (Kunji *et al.*, 1998). *Lactococcus lactis*-derived strains contain a number of different peptidases (at least 9) for the degradation of casein peptides, and many have overlapping activities (Christensen *et al.*, 1999; Liu *et al.*, 2010). Many of the genes or gene clusters for

these proteins/enzymes are plasmid-borne (Siezen *et al.*, 2005). In *L. lactis* SK11, *oppABCDF* and *pepO* are located on one plasmid, while *prtP* is on another (Siezen *et al.*, 2005). During the plasmid curing procedures used to produce the strains *L. lactis* IL1403 (Chopin *et al.*, 1984) and *L. lactis* MG1363 (Gasson, 1983), some of these genes including the essential proteinase PrtP were lost. As a result, these bacteria cannot be grown in milk anymore. It has been proposed that in *L. lactis* dairy isolates, proteases and peptidases provide a selective advantage when growing in milk and this proteolytic system is also found on the chromosome of *S. thermophilus* LMD9 (Liu *et al.*, 2010). The genes are, however, not restricted to dairy isolates, and some plant-derived *L. lactis* strains have the largest set of proteolytic system genes (Liu *et al.*, 2010). Subtle changes in the substrate-binding site(s) can change the substrate specificity of lactococcal PrtP proteinases (Exterkate *et al.*, 1993), and it is therefore possible that the proteolytic system also allows *L. lactis* plant isolates to utilize proteins found in their specific environmental niche. Sequencing of more *L. lactis* plant isolates as well as investigation into the cleavage specificity of the proteinases may indicate whether the PrtP proteinases present in these strains differ in their substrate-binding regions and whether the switch to dairy environments has resulted in the adaptation of PrtP proteinases to casein degradation.

Other HGT events have allowed *S. thermophilus* to adapt to the dairy environment. *Streptococcus thermophilus* possesses a 3.6-kb chromosomal region with extensive similarity to genes from *Lactobacillus bulgaricus*, which enables it to synthesize methionine (Bolotin *et al.*, 2004). There is a high level of sequence similarity (95%) between both species suggesting a recent HGT event. The two species are rather distantly related. They are used together in yoghurt manufacture and have been observed to adhere to each other (Bolotin *et al.*, 2004). Close association could facilitate the gene transfer between *S. thermophilus* and *L. bulgaricus*. The *S. thermophilus* chromosome also contains regions with very high similarity to the genomes of *L. lactis* ssp. *lactis* and *L. lactis* ssp. *cremoris* (Bolotin *et al.*, 2004). These could also have been transferred between the bacteria owing to their close ecological proximity.

Adaptation to a pathogenic lifestyle is, in general, accompanied by genome reduction, and pathogens often have small genomes. This results in the evolution of strictly host-dependent bacteria as the bacteria rely on the host cell to compensate for lost gene functions. Genome reduction, however, can be coupled to the acquisition of genomic islands (GIs) and in particular pathogenicity islands (PAIs) via HGT. PAIs often contain functional genes related to drug resistance, immunity, virulence, and metabolism (reviewed in Dobrindt *et al.*, 2004). Newly acquired PAIs have been linked to recent outbreaks of *S. suis* in China (Chen *et al.*, 2007). An 89-kb PAI was identified, encoding a zeta-toxin, a two-component signal transduction system, three ABC transporter cassettes, and components of a type IV secretion system, which has been demonstrated to mediate HGT of the PAI and contribute to virulence (Li *et al.*, 2011; Zhao *et al.*, 2011).

In bovine-specific *S. agalactiae*, eight GIs were identified. One GI included an operon involved in the production and immunity of the lantibiotic salivaricin A, with high similarity (94.8%) to genes from *S. pyogenes* (Richards *et al.*, 2011). In addition, another GI-containing operon for fructose and lactose utilization exhibits high similarity (99%) to a contiguous sequence in *Streptococcus dysgalactiae* ssp. *dysgalactiae* (Richards *et al.*, 2011). The presence of these GIs indicates that not only has the genome of mastitis-causing *S. agalactiae* strains been shaped by HGT, but specifically by HGT from other streptococcal species that cause mastitis. The ability to utilize lactose and fructose might facilitate the survival of *S. agalactiae* in both mammary and nonmammary organs such as the rumen (Richards *et al.*, 2011). *Streptococcus pneumoniae* has also adapted to use carbon and nitrogen sources specific to the host tissue it invades. In addition to sugars and amino acids or peptides, polysaccharides and hexosamines are important potential nutritional sources for *S. pneumoniae*. The genome of *S. pneumoniae* encodes extracellular enzymatic pathways utilizing *N*-acetylglucosaminidases, α- and β-galactosidases, endoglycosidases, hydrolases, hyaluronidases, and neuraminidases, enabling the degradation of host mucins, glycolipids, and hyaluronic acid and the bacterium's own capsule (Ferretti *et al.*, 2001; Tettelin *et al.*, 2001). The enzymes increase the substrate availability to *S. pneumoniae* and also contribute to the virulence of this species, damaging host tissue and facilitating colonization. The presence of extracellular proteases in *S. mutans* contributes to virulence as well as to nutrition (Cowman *et al.*, 1979; Rosengren & Winblad, 1976). Notwithstanding this, all amino acid biosynthetic pathways were identified in the genome of this bacterium, and it may thus be able to synthesize all of the amino acids (Ajdic *et al.*, 2002).

Competence in *Streptococcaceae*

It has been demonstrated that *L. lactis* is able to acquire genetic material through conjugation and bacteriophage transduction (Gasson, 1983; Gasson *et al.*, 1995; Machielsen *et al.*, 2011). Competence-mediated DNA uptake is yet another mechanism for HGT occurring between bacteria. Natural competence has been well studied in *S. pneumoniae* and *S. thermophilus* (Havarstein *et al.*,

1997; Cvitkovitch, 2001), but other streptococci such as *Streptococcus aginosus*, *S. mitis*, and *S. mutans* have also been shown to be naturally transformable (Havarstein *et al.*, 1997; Martin *et al.*, 2006). Interestingly, the discovery of DNA as the hereditary material of life was because of the experiments carried out with *S. pneumoniae*. The observation that heat-treated virulent strains did not kill mice but a mixture of heat-treated virulent and live nonvirulent *S. pneumoniae* strains did by Griffith in 1928 was further investigated by Avery and led to the identification of DNA as the 'transforming principle' in the virulence experiments (see O'Connor (2008) for a review of the early experiments).

Competence development in streptococci relies on the transcriptional induction of *comX*, which encodes the alternative sigma factor σ^x. In *S. pneumoniae*, competence development depends on an early step involving a quorum-sensing system encoded by *comABCDE* (Claverys & Havarstein, 2002). The inducer peptide, encoded by *comC*, is secreted into the extracellular medium by the ABC-type transporter encoded by *comAB* (Alloing *et al.*, 1998). The expression of *comC* increases with cell density, and once its extracellular concentration reaches a critical threshold, the inducer peptide activates the membrane-located histidine kinase ComD, which in turn stimulates the phosphorylation of the response regulator ComE (Cheng *et al.*, 1997). Phosphorylated ComE positively regulates the expression of *comABCDE* as well as *comX* (Pestova *et al.*, 1996). The alternative sigma factor σ^x associates with the RNA polymerase, allowing synthesis of the late competence genes. The late genes encode the DNA uptake machinery, single-stranded DNA-binding proteins as well as proteins essential for homologous recombination (Lee & Morrison, 1999). This cascade also induces competence in *S. mutans* where the early competence genes are *blpABCHR* (Li *et al.*, 2002). In *S. thermophilus* and *S. salivarius*, however, induction of σ^x expression relies on another quorum-sensing system involving the transcriptional regulator ComR and a small hydrophobic peptide pheromone encoded by *comS* (Fontaine *et al.*, 2010). The Ami or Opp oligopeptide transporter plays an essential role in competence development in *S. thermophilus* by reimporting secreted, matured ComS (Gardan *et al.*, 2009; Fontaine *et al.*, 2010). ComS is then postulated to bind to ComR which in turn binds to the *comX* promoter, promoting σ^x expression (Fontaine *et al.*, 2010).

Another major difference between competence development in *S. pneumoniae* and *S. thermophilus* is that while *S. thermophilus* strain LMD-9 can spontaneously turn on competence in chemically defined medium, competence induction in *S. pneumoniae* appears to be stress related (Gardan *et al.*, 2009; Claverys *et al.*, 2006). Even though many streptococci are naturally competent, competence is not a constant property. Rather, it is a transient state regulated by the quorum-sensing mechanisms described above. Yet to be identified optimal conditions for competence may partly explain why, although the genome sequences of many streptococci contain competence genes, not all have been shown to be naturally competent.

Genes for competence development have been identified in *L. lactis* strains, but none of these strains have been naturally transformed. While ComX has been identified and shown to regulate the expression of late competence genes, there is a high occurrence (50% of strains tested) of a mutated *dprA* gene in *L. lactis* ssp. *lactis* strains, including *L. lactis* ssp. *lactis* IL1403 (Wydau *et al.*, 2006). *dprA* (*smf* in *B. subtilis* and *L. lactis* ssp. *cremoris*) encodes for a DNA-processing protein, which has been shown to play an essential role in the fate of DNA taken up during competence in *S. pneumoniae* (Bergé *et al.*, 2003). In an *S. pneumoniae dprA* mutant, the incoming DNA was degraded and DNA fragments were randomly integrated into the chromosome (Bergé *et al.*, 2003). Four different mutations have been identified in the *dprA* genes of *L. lactis* ssp. *lactis* strains, all of which lead to a premature stop codon, inactivating the protein (Wydau *et al.*, 2006). In this background, *L. lactis* may use the DNA uptake machinery to provide DNA to be degraded, increasing the intracellular pool of nucleotides. This has been observed in *E. coli* (Finkel & Kolter, 2001), and as free bases are present in only limited amounts in milk, DNA uptake may provide a growth advantage to *L. lactis*.

Future perspectives

The rapid and increasingly cost-effective development of whole-genome sequencing now allows the in-depth analysis of many *L. lactis* ssp. and *Streptococcus* species and serotypes rather than type strains only. Sequencing of *Streptococcaceae* important to the dairy industry has revealed that the switch to nutritionally rich environments has been instrumental in the shaping of these small genomes and, importantly, the absence of virulence genes in the genome *S. thermophilus* has cemented its GRAS status.

The molecular mechanisms underlying the switch from homolactic to mixed-acid fermentation in *L. lactis* are still unresolved. Because the end products of fermentation have an important influence on the flavor of fermented food products, manipulation of flux through these pathways would allow a targeted approach to flavor formation in products such as cheese and yoghurt. Resequencing of industrial strains would also contribute to the elucidation of specific pathways that contribute to the desirable and nondesirable flavors in existing dairy products.

Exploitation of the natural diversity in carbohydrate and protein utilization systems in lactococci may allow for the development of new, possibly nondairy, fermented food products. Also, engineering of existing acid tolerance systems and cold-shock proteins in dairy *Streptococcaceae* may produce more robust starter cultures.

Beyond this, the wealth of information generated with regard to virulence genes of pathogenic and nonpathogenic *Streptococcus* serotypes sequenced to date will lead to more targeted treatments of the numerous life-threatening diseases caused by these bacteria.

References

Abbe K, Carlsson J, Takahashi-Abbe S & Yamada T (1991) Oxygen and the sugar metabolism in oral streptococci. *Proc Finn Dent Soc* **87**: 477–487.

Abdelal AT (1979) Arginine catabolism by microorganisms. *Annu Rev Microbiol* **33**: 139–168.

Abranches J, Nascimento MM, Zeng L, Browngardt CM, Wen ZT, Rivera MF & Burne RA (2008) CcpA regulates central metabolism and virulence gene expression in *Streptococcus mutans*. *J Bacteriol* **190**: 2340–2349.

Ajdic D & Pham VTT (2007) Global transcriptional analysis of *Streptococcus mutans* sugar transporters using microarrays. *J Bacteriol* **189**: 5049–5059.

Ajdic D, McShan WM, McLaughlin RE *et al.* (2002) Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *P Natl Acad Sci USA* **99**: 14434–14439.

Alloing G, Martin B, Granadel C & Claverys J-P (1998) Development of competence in *Streptococcus pneumoniae*: pheromone autoinduction and control of quorum sensing by the oligopeptide permease. *Mol Microbiol* **29**: 75–83.

Anders RF, Hogg DM & Jago GR (1970) Formation of hydrogen peroxide by Group N streptococci and its effect on their growth and metabolism. *Appl Environ Microbiol* **19**: 608–612.

Andersen HW, Solem C, Hammer K & Jensen PR (2001) Twofold reduction of phosphofructokinase activity in *Lactococcus lactis* results in strong decreases in Growth rate and in glycolytic flux. *J Bacteriol* **183**: 3458–3467.

Auzat I, Chapuy-Regaud S, Le Bras G, Dos Santos D, Ogunniyi AD, Le Thomas I, Garel J-R, Paton JC & Trombe M-C (1999) The NADH oxidase of *Streptococcus pneumoniae*: its involvement in competence and virulence. *Mol Microbiol* **34**: 1018–1028.

Bachmann H (2009) *Regulatory and Adaptive Responses of Lactococcus lactis in situ*. Wageningen University, Wageningen, the Netherlands.

Bender GR, Sutton SV & Marquis RE (1986) Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. *Infect Immun* **53**: 331–338.

Bergé M, Mortier-Barrière I, Martin B & Claverys J-P (2003) Transformation of *Streptococcus pneumoniae* relies on DprA- and RecA-dependent protection of incoming DNA single strands. *Mol Microbiol* **50**: 527–536.

Bolotin A, Wincker P, Mauger S, Jaillon O, Malarme K, Weissenbach J, Ehrlich SD & Sorokin A (2001) The complete genome sequence of the lactic acid bacterium *Lactococcus lactis* ssp. *lactis* IL1403. *Genome Res* **11**: 731–753.

Bolotin A, Quinquis B, Renault P *et al.* (2004) Complete sequence and comparative genome analysis of the dairy bacterium *Streptococcus thermophilus*. *Nat Biotechnol* **22**: 1554–1558.

Boucher I, Vadeboncoeur C & Moineau S (2003) Characterization of genes involved in the metabolism of α-galactosides by *Lactococcus raffinolactis*. *Appl Environ Microbiol* **69**: 4049–4056.

Brooijmans RJW, Poolman B, Schuurman-Wolters GK, de Vos WM & Hugenholtz J (2007) Generation of a membrane potential by *Lactococcus lactis* through aerobic electron transport. *J Bacteriol* **189**: 5203–5209.

Brown AT & Wittenberger CL (1973) Mannitol and sorbitol catabolism in *Streptococcus mutans*. *Arch Oral Biol* **18**: 117–125.

Bryan-Jones DG & Whittenbury R (1969) Haematin-dependent oxidative phosphorylation in *Streptococcus faecalis*. *J Gen Microbiol* **58**: 247–260.

Cai Y, Yang J, Pang H & Kitahara M (2011) *Lactococcus fujiensis* sp *nov*., a lactic acid bacterium isolated from vegetable. *Int J Syst Evol Microbiol* **61**: 1590–1594.

Calmes R (1978) Involvement of phosphoenolpyruvate in the catabolism of caries-conducive disaccharides by *Streptococcus mutans*: lactose transport. *Infect Immun* **19**: 934–942.

Casiano-Colón A & Marquis RE (1988) Role of the arginine deiminase system in protecting oral bacteria and an enzymatic basis for acid tolerance. *Appl Environ Microbiol* **54**: 1318–1324.

Castro R, Neves AR, Fonseca LL, Pool W, Kok J, Kuipers OP & Santos H (2009) Characterization of the individual glucose uptake systems of *Lactococcus lactis*: mannose-PTS, cellobiose-PTS and the novel GlcU permease. *Mol Microbiol* **71**: 795–806.

Charrier V, Buckley E, Parsonage D, Galinier A, Darbon E, Jaquinod M, Forest E, Deutscher J & Claiborne A (1997) Cloning and sequencing of two enterococcal *glpK* genes and regulation of the encoded glycerol kinases by phosphoenolpyruvate-dependent, phosphotransferase system-catalyzed phosphorylation of a single histidyl residue. *J Biol Chem* **272**: 14166–14174.

Chassy BM & Porter EV (1979) Initial characterization of sucrose-6-phosphate hydrolase from and its apparent identity with intracellular invertase. *Biochem Biophys Res Commun* **89**: 307–314.

Chen C, Tang J, Dong W *et al.* (2007) A glimpse of streptococcal toxic shock syndrome from comparative genomics of *S. suis* 2 Chinese isolates. *PLoS ONE* **2**: 315.

Cheng Q, Campbell EA, Naughton AM, Johnson S & Masure HR (1997) The *com* locus controls genetic transformation in *Streptococcus pneumoniae*. *Mol Microbiol* **23**: 683–692.

Cho S-L, Nam S-W, Yoon J-H, Lee J-S, Sukhoom A & Kim W (2008) *Lactococcus chungangensis* sp. *nov*., a lactic acid

bacterium isolated from activated sludge foam. *Int J Syst Evol Microbiol* **58**: 1844–1849.

Chopin A, Chopin MC, Moillo-Batt A & Langella P (1984) Two plasmid-determined restriction and modification systems in *Streptococcus lactis*. *Plasmid* **11**: 260–263.

Christensen JE, Dudley EG, Pederson JA & Steele JL (1999) Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie Van Leeuwenhoek* **76**: 217–246.

Chritstensen KR & Reineccius GA (1995) Aroma extract dilution analysis of aged cheddar cheese. *J Food Sci* **60**: 218–220.

Claverys JP & Havarstein LS (2002) Extracellular-peptide control of competence for genetic transformation in *Streptococcus pneumoniae*. *Front Biosci* **7**: 1798–1814.

Claverys J-P, Prudhomme M & Martin B (2006) Induction of competence regulons as a general response to stress in gram-positive bacteria. *Annu Rev Microbiol* **60**: 451–475.

Cocaign-Bousquet M, Garrigues C, Loubiere P & Lindley ND (1996) Physiology of pyruvate metabolism in *Lactococcus lactis*. *Antonie Van Leeuwenhoek* **70**: 253–267.

Cogan TM & Hill C (1993) Cheese starter cultures. *Cheese: Chemistry, Physics and Microbiology*, Vol **I** (Fox PF, ed), pp. 193–250. Chapman & Hall, London, UK.

Cotter PD, Hill C & Ross RP (2005) Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* **3**: 777–788.

Cowman RA, Schaefer SJ, Fitzgerald RJ, Rosner D, Shklair IL & Walter RG (1979) Differential utilization of proteins in saliva from caries-active and caries-free subjects as growth substrates by plaque-forming streptococci. *J Dent Sci* **58**: 2019–2027.

Crow VL & Thomas TD (1982) Arginine metabolism in lactic streptococci. *J Bacteriol* **150**: 1024–1032.

Cunin R, Glansdorff N, Pierard A & Stalon V (1986) Biosynthesis and metabolism of arginine in bacteria. *Microbiol Rev* **50**: 314–352.

Curioni PMG & Bosset JO (2002) Key odorants in various cheese types as determined by gas chromatography-olfactometry. *Int Dairy J* **12**: 959–984.

Cvitkovitch DG (2001) Genetic competence and transformation in oral streptococci. *Crit Rev Oral Biol Med* **12**: 217–243.

Cvitkovitch DG, Gutierrez JA & Bleiweis AS (1997) Role of the citrate pathway in glutamate biosynthesis by *Streptococcus mutans*. *J Bacteriol* **179**: 650–655.

Dacremont C & Vickers Z (1994) Concept matching technique for assessing importance of volatile compounds for cheddar cheese aroma. *J Food Sci* **59**: 981–985.

De Felipe FL & Hugenholtz J (1999) Pyruvate flux distribution in NADH-oxidase-overproducing *Lactococcus lactis* strain as a function of culture conditions. *FEMS Microbiol Lett* **179**: 461–466.

De Felipe F, Starrenburg MJC & Hugenholtz J (1997) The role of NADH-oxidation in acetoin and diacetyl production from glucose in *Lactococcus lactis* subsp. *lactis* MG1363. *FEMS Microbiol Lett* **156**: 15–19.

De Felipe F, Kleerebezem M, de Vos WM & Hugenholtz J (1998) Cofactor engineering: a novel approach to metabolic engineering in *Lactococcus lactis* by controlled expression of NADH oxidase. *J Bacteriol* **180**: 3804–3808.

de Vos WM & Vaughan EE (1994) Genetics of lactose utilization in lactic acid bacteria. *FEMS Microbiol Rev* **15**: 217–237.

Deibel RH (1964) Utilization of arginine as an energy source for the growth of *Streptococcus faecalis*. *J Bacteriol* **87**: 988–992.

Delorme C, Godon JJ, Ehrlich SD & Renault P (1993) Gene inactivation in *Lactococcus lactis*: histidine biosynthesis. *J Bacteriol* **175**: 4391–4399.

Deutscher J, Küster E, Bergstedt U, Charrier V & Hillen W (1995) Protein kinase-dependent HPr/CcpA interaction links glycolytic activity to carbon catabolite repression in Gram-positive bacteria. *Mol Microbiol* **15**: 1049–1053.

Dobrindt U, Hochhut B, Hentschel U & Hacker J (2004) Genomic islands in pathogenic and environmental microorganisms. *Nat Rev Micro* **2**: 414–424.

Dong Y, Chen Y-YM & Burne RA (2004) Control of expression of the arginine deiminase operon of *Streptococcus gordonii* by CcpA and Flp. *J Bacteriol* **186**: 2511–2514.

Dossonnet V, Monedero V, Zagorec M, Galinier A, Pérez-Martínez G & Deutscher J (2000) Phosphorylation of HPr by the bifunctional HPr kinase/P-Ser-HPr phosphatase from *Lactobacillus casei* controls catabolite repression and inducer exclusion but not inducer expulsion. *J Bacteriol* **182**: 2582–2590.

Duwat P, Sourice S, Cesselin B, Lamberet G, Vido K, Gaudu P, Le Loir Y, Violet F, Loubiére P & Gruss A (2001) Respiration capacity of the fermenting bacterium *Lactococcus lactis* and its positive effects on growth and survival. *J Bacteriol* **183**: 4509–4516.

Duwat P, Ehrlich SD & Gruss A (1995) The *recA* gene of *Lactococcus lactis*: characterization and involvement in oxidative and thermal stress. *Mol Microbiol* **17**: 1121–1131.

Escamilla-Hurtado ML, Tomasini-Campocosio A, Valdés-Martínez S & Soriano-Santos J (1996) Diacetyl formation by lactic bacteria. *Rev Latinoam Microbiol* **38**: 129–137.

Esgleas M, Li Y, Hancock MA, Harel J, Dubreuil JD & Gottschalk M (2008) Isolation and characterization of α-enolase, a novel fibronectin-binding protein from *Streptococcus suis*. *Microbiology* **154**: 2668–2679.

Evershed RP, Payne S, Sherratt AG *et al.* (2008) Earliest date for milk use in the Near East and southeastern Europe linked to cattle herding. *Nature* **455**: 528–531.

Exterkate FA, Alting AC & Bruinenberg PG (1993) Diversity of cell envelope proteinase specificity among strains of *Lactococcus lactis* and its relationship to charge characteristics of the substrate-binding region. *Appl Environ Microbiol* **59**: 3640–3647.

Ferretti JJ, McShan WM, Ajdic D *et al.* (2001) Complete genome sequence of an M1 strain of *Streptococcus pyogenes*. *P Natl Acad Sci USA* **98**: 4658–4663.

Finkel SE & Kolter R (2001) DNA as a nutrient: novel role for bacterial competence gene homologs. *J Bacteriol* **183**: 6288–6293.

Fontaine L, Boutry C, de Frahan MH, Delplace B, Fremaux C, Horvath P, Boyaval P & Hols P (2010) A novel pheromone quorum-sensing system controls the development of natural competence in *Streptococcus thermophilus* and *Streptococcus salivarius*. *J Bacteriol* **192**: 1444–1454.

Gardan R, Besset C, Guillot A, Gitton C & Monnet V (2009) The oligopeptide transport system is essential for the development of natural competence in *Streptococcus thermophilus* strain LMD-9. *J Bacteriol* **191**: 4647–4655.

Garrigues C, Loubiere P, Lindley ND & Cocaign-Bousquet M (1997) Control of the shift from homolactic acid to mixed-acid fermentation in *Lactococcus lactis*: predominant role of the NADH/NAD+ ratio. *J Bacteriol* **179**: 5282–5287.

Gaspar P, Neves AR, Shearman CA, Gasson MJ, Baptista AM, Turner DL, Soares CM & Santos H (2007) The lactate dehydrogenases encoded by the *ldh* and *ldhB* genes in *Lactococcus lactis* exhibit distinct regulation and catalytic properties – comparative modeling to probe the molecular basis. *FEBS J* **274**: 5924–5936.

Gasson MJ (1983) Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. *J Bacteriol* **154**: 1–9.

Gasson MJ, Godon JJ, Pillidge CJ, Eaton TJ, Jury K & Shearman CA (1995) Characterization and exploitation of conjugation in *Lactococcus lactis*. *Int Dairy J* **5**: 757–762.

Gaudu P, Lamberet G, Poncet S & Gruss A (2003) CcpA regulation of aerobic and respiration growth in *Lactococcus lactis*. *Mol Microbiol* **50**: 183–192.

Gauthier L, Mayrand D & Vadeboncoeur C (1984a) Isolation of a novel protein involved in the transport of fructose by an inducible phosphoenolpyruvate fructose phosphotransferase system in *Streptococcus mutans*. *J Bacteriol* **160**: 755–763.

Gauthier L, Vadeboncœur C & Mayrand D (1984b) Loss of sensitivity to xylitol by *Streptococcus mutans* LG-1. *Caries Res* **18**: 289–295.

Gauthier M, Brochu D, Eltis LD, Thomas S & Vadeboncoeur C (1997) Replacement of isoleucine-47 by threonine in the HPr protein of *Streptococcus salivarius* abrogates the preferential metabolism of glucose and fructose over lactose and melibiose but does not prevent the phosphorylation of HPr on serine-46. *Mol Microbiol* **25**: 695–705.

Gibello A, Collins MD, Dominguez L, Fernandez-Garayzabal JF & Richardson PT (1999) Cloning and analysis of the L-lactate utilization genes from *Streptococcus iniae*. *Appl Environ Microbiol* **65**: 4346–4350.

Gibson CM, Mallett TC, Claiborne A & Caparon MG (2000) Contribution of NADH oxidase to aerobic metabolism of *Streptococcus pyogenes*. *J Bacteriol* **182**: 448–455.

Gilliland SE (1989) Acidophilus milk products: a review of potential benefits to consumers. *J Dairy Sci* **72**: 2483–2494.

Godon JJ, Delorme C, Bardowski J, Chopin MC, Ehrlich SD & Renault P (1993) Gene inactivation in *Lactococcus lactis*: branched-chain amino acid biosynthesis. *J Bacteriol* **175**: 4383–4390.

Goffin P, Muscariello L, Lorquet F, Stukkens A, Prozzi D, Sacco M, Kleerebezem M & Hols P (2006) Involvement of pyruvate oxidase activity and acetate production in the survival of *Lactobacillus plantarum* during the stationary phase of aerobic growth. *Appl Environ Microbiol* **72**: 7933–7940.

Górecki RK, Koryszewska-Bagińska A, Gołębiewski M, Żylińska J, Grynberg M & Bardowski JK (2011) Adaptative potential of the *Lactococcus Lactis* IL594 strain encoded in its 7 plasmids. *PLoS ONE* **6**: e22238.

Grossiord BP, Luesink EJ, Vaughan EE, Arnaud A & de Vos WM (2003) Characterization, expression, and mutation of the *Lactococcus lactis galPMKTE* genes, involved in galactose utilization via the Leloir pathway. *J Bacteriol* **185**: 870–878.

Gunnewijk MGW, Postma PW & Poolman B (1999) Phosphorylation and functional properties of the IIA domain of the lactose transport protein of *Streptococcus thermophilus*. *J Bacteriol* **181**: 632–641.

Han X, Ding C, Chen H, Hu Q & Yu S (2011) Enzymatic and biological characteristics of enolase in *Brucella abortus* A19. *Mol Biol Rep* DOI 10.1007/s11033-011-1025-6.

Hansson L & Häggström MH (1984) Effects of growth conditions on the activities of superoxide dismutase and NADH-oxidase/NADH-peroxidase *Streptococcus lactis*. *Curr Microbiol* **10**: 345–351.

Harold FM (1986) *The Vital Force: A Study of Bioenergetics*. W.H. Freeman, New York.

Havarstein LS, Hakenbeck R & Gaustad P (1997) Natural competence in the genus *Streptococcus*: evidence that streptococci can change phenotype by interspecies recombinational exchanges. *J Bacteriol* **179**: 6589–6594.

Henriksen CM & Nilsson D (2001) Redirection of pyruvate catabolism in *Lactococcus lactis* by selection of mutants with additional growth requirements. *Appl Microbiol Biotechnol* **56**: 767–775.

Higuchi M, Shimada M, Yamamoto Y, Hayashi T, Koga T & Kamio Y (1993) Identification of two distinct NADH oxidases corresponding to H_2O_2-forming oxidase and H_2O-forming oxidase induced in *Streptococcus mutans*. *J Gen Microbiol* **139**: 2343–2351.

Higuchi M, Yamamoto Y, Poole LB, Shimada M, Sato Y, Takahashi N & Kamio Y (1999) Functions of two types of NADH oxidases in energy metabolism and oxidative stress of *Streptococcus mutans*. *J Bacteriol* **181**: 5940–5947.

Hillier AJ & Jago GR (1982) L-Lactate dehydrogenase, FDP-activated, from *Streptococcus cremoris*. *Methods Enzymol* **89**: 362–367.

Holzapfel WH & Wood BJB (1995) Lactic acid bacteria in contemporary perspective. *Genera of Lactic Acid Bacteria* (Wood BJB & Holzapfel 1995, eds), pp. 1–6. Blackie Academic & Professional, London, UK.

Jamet E, Ehrlich SD, Duperray F & Renault P (2001) Study of the duplicated glycolytic genes in *Lactococcus lactis* IL1403. *Lait* **81**: 115–129.

Jeanson S, Hilgert N, Coquillard M-O *et al.* (2009) Milk acidification by *Lactococcus lactis* is improved by decreasing the level of dissolved oxygen rather than decreasing redox

potential in the milk prior to inoculation. *Int J Food Microbiol* **131**: 75–81.

Jensen NBS, Melchiorsen CR, Jokumsen KV & Villadsen J (2001) Metabolic behavior of *Lactococcus lactis* MG1363 in microaerobic continuous cultivation at a low dilution rate. *Appl Environ Microbiol* **67**: 2677–2682.

Juillard V, Le Bars D, Kunji ER, Konings WN, Gripon JC & Richard J (1995) Oligopeptides are the main source of nitrogen for *Lactococcus lactis* during growth in milk. *Appl Environ Microbiol* **61**: 3024–3030.

Kandler O (1983) Carbohydrate metabolism in lactic acid bacteria. *Antonie Van Leeuwenhoek* **49**: 209–224.

Karlin S, Theriot J & Mrázek J (2004) Comparative analysis of gene expression among low G+C gram-positive genomes. *P Natl Acad Sci USA* **101**: 6182–6187.

Kelly WJ, Ward LJH & Leahy SC (2010) Chromosomal diversity in *Lactococcus lactis* and the origin of dairy starter cultures. *Genome Biol Evol* **2**: 729–744.

Kempler GM & McKay LL (1981) Biochemistry and genetics of citrate utilization in *Streptococcus lactis* ssp. *diacetylactis*. *J Dairy Sci* **64**: 1527–1539.

Kenney EB & Ash MMJ (1969) Oxidation reduction potential of developing plaque, periodontal pockets and gingival sulci. *J Periodontol* **40**: 630–633.

Koebmann B, Andersen H, Solem C & Jensen P (2002) Experimental determination of control of glycolysis in *Lactococcus lactis*. *Antonie Van Leeuwenhoek* **82**: 237–248.

Konings WN (2002) The cell membrane and the struggle for life of lactic acid bacteria. *Antonie Van Leeuwenhoek* **82**: 3–27.

Konings WN, Poolman B, Driessen AJM & Maloney PC (1989) Bioenergetics and solute transport in lactococci. *Crit Rev Microbiol* **16**: 419–476.

Kunji ERS, Fang G, Jeronimus-Stratingh CM, Bruins AP, Poolman B & Konings WN (1998) Reconstruction of the proteolytic pathway for use of β-casein by *Lactococcus lactis*. *Mol Microbiol* **27**: 1107–1118.

Larsen R, Kok J & Kuipers OP (2005) Interaction between ArgR and AhrC controls regulation of arginine metabolism in *Lactococcus lactis*. *J Biol Chem* **280**: 19319–19330.

Lee MS & Morrison DA (1999) Identification of a new regulator in *Streptococcus pneumoniae* linking quorum sensing to competence for genetic transformation. *J Bacteriol* **181**: 5004–5016.

Li Y-H, Tang N, Aspiras MB, Lau PCY, Lee JH, Ellen RP & Cvitkovitch DG (2002) A quorum-sensing signaling system essential for genetic competence in *Streptococcus mutans* is involved in biofilm formation. *J Bacteriol* **184**: 2699–2708.

Li M, Shen X, Yan J *et al.* (2011) GI-type T4SS-mediated horizontal transfer of the 89K pathogenicity island in epidemic *Streptococcus suis* serotype 2. *Mol Microbiol* **79**: 1670–1683.

Liu Y, Dong Y, Chen Y-YM & Burne RA (2008) Environmental and growth phase regulation of the *Streptococcus gordonii* arginine deiminase genes. *Appl Environ Microbiol* **74**: 5023–5030.

Liu M, Bayjanov J, Renckens B, Nauta A & Siezen R (2010) The proteolytic system of lactic acid bacteria revisited: a genomic comparison. *BMC Genomics* **11**: 36.

Loubiere P, Cocaign-Bousquet M, Matos J, Goma G & Lindley ND (1997) Influence of end-products inhibition and nutrient limitations on the growth of *Lactococcus lactis* subsp. *lactis*. *J Appl Microbiol* **82**: 95–100.

Luesink EJ, van Herpen R, Grossiord BP, Kuipers OP & de Vos WM (1998) Transcriptional activation of the glycolytic *las* operon and catabolite repression of the *gal* operon in *Lactococcus lactis* are mediated by the catabolite control protein CcpA. *Mol Microbiol* **30**: 789–798.

Luesink EJ, Beumer CM, Kuipers OP & de Vos WM (1999) Molecular characterization of the *Lactococcus lactis* ptsHI operon and analysis of the regulatory role of HPr. *J Bacteriol* **181**: 764–771.

Machielsen R, Siezen RJ, van Hijum SAFT & van Hylckama Vlieg JET (2011) Molecular description and industrial potential of Tn6098 conjugative transfer conferring α-galactoside metabolism in *Lactococcus lactis*. *Appl Environ Microbiol* **77**: 555–563.

Makarova KS & Koonin EV (2007) Evolutionary genomics of lactic acid bacteria. *J Bacteriol* **189**: 1199–1208.

Makarova K, Slesarev A, Wolf Y *et al.* (2006) Comparative genomics of the lactic acid bacteria. *P Natl Acad Sci USA* **103**: 15611–15616.

Maloney PC (1982) Energy coupling to ATP synthesis by the proton-translocating ATPase. *J Membr Biol* **67**: 1–12.

Martin B, Quentin Y, Fichant G & Claverys J-P (2006) Independent evolution of competence regulatory cascades in streptococci? *Trends Microbiol* **14**: 339–345.

Maryanski JH & Wittenberger CL (1975) Mannitol transport in *Streptococcus mutans*. *J Bacteriol* **124**: 1475–1481.

Mason EO, Wald ER, Tan TQ, Schutze GE, Bradley JS, Barson WJ, Givner LB, Hoffman J & Kaplan SL (2007) Recurrent systemic pneumococcal disease in children. *Pediatr Infect Dis J* **26**: 480–484.

McClelland M, Sanderson KE, Spieth J *et al.* (2001) Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature* **413**: 852–856.

Medina M, Vintiñi E, Villena J, Raya R & Alvarez S (2010) *Lactococcus lactis* as an adjuvant and delivery vehicle of antigens against pneumococcal respiratory infections. *Bioeng Bugs* **1**: 313–325.

Melchiorsen CR, Jokumsen KV, Villadsen J, Johnsen MG, Israelsen H & Arnau J (2000) Synthesis and posttranslational regulation of pyruvate formate-lyase in *Lactococcus lactis*. *J Bacteriol* **182**: 4783–4788.

Michels PAM, Michels JPJ, Boonstra J & Konings WN (1979) Generation of an electrochemical proton gradient in bacteria by the excretion of metabolic end products. *FEMS Microbiol Lett* **5**: 357–364.

Mickelson MN (1972) Glucose degradation, molar growth yields, and evidence for oxidative phosphorylation in *Streptococcus agalactiae*. *J Bacteriol* **109**: 96–105.

Mills OE & Thomas TD (1981) Nitrogen sources for growth of lactic streptococci in milk. *N Z J Dairy Sci Technol* **16**: 43–55.

Miyoshi A, Rochat T, Gratadoux J-J, Le Loir Y, Oliveira SC, Langella P & Azevedo V (2003) Oxidative stress in *Lactococcus lactis*. *Genet Mol Res* **2**: 348–359.

Monedero V, Kuipers OP, Jamet E & Deutscher J (2001a) Regulatory functions of serine-46-phosphorylated HPr in *Lactococcus lactis*. *J Bacteriol* **183**: 3391–3398.

Monedero V, Poncet S, Mijakovic I, Fieulaine S, Dossonnet V, Martin-Verstraete I, Nessler S & Deutscher J (2001b) Mutations lowering the phosphatase activity of HPr kinase/phosphatase switch off carbon metabolism. *EMBO J* **20**: 3928–3937.

Mufson M (1999) Pneumococcal pneumonia. *Curr Infect Dis Rep* **1**: 57–64.

Munro C, Michalek SM & Macrina FL (1991) Cariogenicity of *Streptococcus mutans* V403 glucosyltransferase and fructosyltransferase mutants constructed by allelic exchange. *Infect Immun* **59**: 2316–2323.

Musher DM (1992) Infections caused by *Streptococcus pneumoniae*: clinical spectrum, pathogenesis, immunity, and treatment. *Clin Infect Dis* **14**: 801–809.

Naumoff D, Xu Y, Glansdorff N & Labedan B (2004) Retrieving sequences of enzymes experimentally characterized but erroneously annotated: the case of the putrescine carbamoyltransferase. *BMC Genomics* **5**: 52.

Néron S & Vadeboncoeur C (1987) Evidence for the presence of two distinct phosphoenolpyruvate: mannose phosphotransferase systems in *Streptococcus mutans* GS5-2. *FEMS Microbiol Lett* **42**: 7–11.

Neves AR, Ramos A, Costa H, van Swam II, Hugenholtz J, Kleerebezem M, de Vos W & Santos H (2002a) Effect of different NADH oxidase levels on glucose metabolism by *Lactococcus lactis*: kinetics of intracellular metabolite pools determined by *in vivo* nuclear magnetic resonance. *Appl Environ Microbiol* **68**: 6332–6342.

Neves AR, Ventura R, Mansour N, Shearman C, Gasson MJ, Maycock C, Ramos A & Santos H (2002b) Is the glycolytic flux in *Lactococcus lactis* primarily controlled by the redox charge? *J Biol Chem* **277**: 28088–28098.

Newbrun E (1983) *Cariology*. Williams & Wilkins, Baltimore, MD.

Niven CF, Smiley KL & Sherman JM (1941) The hydrolysis of arginine by streptococci. *J Bacteriol* **43**: 651–660.

Novak L & Loubiere P (2000) The metabolic network of *Lactococcus lactis*: distribution of ^{14}C-labeled substrates between catabolic and anabolic pathways. *J Bacteriol* **182**: 1136–1143.

O'Connor C (2008) Discovery of DNA as the hereditary material using *Streptococcus pneumoniae*. Nat Education 1.

Otto R, Hugenholtz J, Konings WN & Veldkamp H (1980a) Increase of molar growth yield of *Streptococcus cremoris* for lactose as a consequence of lactate consumption by *Pseudomonas stutzeri* in mixed culture. *FEMS Microbiol Lett* **9**: 85–88.

Otto R, Sonnenberg AS, Veldkamp H & Konings WN (1980b) Generation of an electrochemical proton gradient in *Streptococcus cremoris* by lactate efflux. *P Natl Acad Sci USA* **77**: 5502–5506.

Pastink MI, Teusink B, Hols P, Visser S, de Vos WM & Hugenholtz J (2009) Genome-scale model of *Streptococcus thermophilus* LMG18311 for metabolic comparison of lactic acid bacteria. *Appl Environ Microbiol* **75**: 3627–3633.

Perez T, Balcazar JL, Peix A, Valverde A, Velazquez E, de Blas I & Ruiz-Zarzuela I (2011) *Lactococcus lactis* subsp. *tructae* subsp. *nov*. isolated from the intestinal mucus of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). *Int J Syst Evol Microbiol* **61**: 1894–1898.

Pericone CD, Overweg K, Hermans PWM & Weiser JN (2000) Inhibitory and bactericidal effects of hydrogen peroxide production by *Streptococcus pneumoniae* on other inhabitants of the upper respiratory tract. *Infect Immun* **68**: 3990–3997.

Pericone CD, Park S, Imlay JA & Weiser JN (2003) Factors contributing to hydrogen peroxide resistance in *Streptococcus pneumoniae* include pyruvate oxidase (SpxB) and avoidance of the toxic effects of the Fenton reaction. *J Bacteriol* **185**: 6815–6825.

Pestova EV, Håvarstein LS & Morrison DA (1996) Regulation of competence for genetic transformation in *Streptococcus pneumoniae* by an auto-induced peptide pheromone and a two-component regulatory system. *Mol Microbiol* **21**: 853–862.

Poolman B (1993) Energy transduction in lactic acid bacteria. *FEMS Microbiol Rev* **12**: 125–147.

Poolman B & Konings WN (1988) Relation of growth of *Streptococcus lactis* and *Streptococcus cremoris* to amino acid transport. *J Bacteriol* **170**: 700–707.

Poolman B, Driessen AJ & Konings WN (1987) Regulation of arginine-ornithine exchange and the arginine deiminase pathway in *Streptococcus lactis*. *J Bacteriol* **169**: 5597–5604.

Poolman B, Molenaar D, Smid EJ, Ubbink T, Abee T, Renault PP & Konings WN (1991) Malolactic fermentation: electrogenic malate uptake and malate/lactate antiport generate metabolic energy. *J Bacteriol* **173**: 6030–6037.

Postma PW, Lengeler JW & Jacobson GR (1993) Phosphoenolpyruvate:carbohydrate phosphotransferase systems of bacteria. *Microbiol Rev* **57**: 543–594.

Poy F & Jacobson GR (1990) Evidence that a low-affinity sucrose phosphotransferase activity in *Streptococcus mutans* GS-5 is a high-affinity trehalose uptake system. *Infect Immun* **58**: 1479–1480.

Quivey RG, Kuhnert WL & Hahn K (2000) Adaptation of oral streptococci to low pH. *Adv Microb Physiol* **42**: 239–274.

Quivey RG, Kuhnert WL & Hahn K (2001) Genetics of acid adaptation in oral streptococci. *Crit Rev Oral Biol Med* **12**: 301–314.

Regev-Yochay G, Trzcinski K, Thompson CM, Lipsitch M & Malley R (2007) SpxB is a suicide gene of *Streptococcus pneumoniae* and confers a selective advantage in an *in vivo* competitive colonization model. *J Bacteriol* **189**: 6532–6539.

Reizer J & Panos C (1980) Regulation of β-galactoside phosphate accumulation in *Streptococcus pyogenes* by an expulsion mechanism. *P Natl Acad Sci USA* **77**: 5497–5501.

Reizer J & Saier MHJ (1983) Involvement of lactose enzyme II of the phosphotransferase system in rapid expulsion of free galactosides from *Streptococcus pyogenes*. *J Bacteriol* **156**: 236–242.

Reizer J, Novotny MJ, Panos C & Saier MHJ (1983) Mechanism of inducer expulsion in *Streptococcus pyogenes*: a two-step process activated by ATP. *J Bacteriol* **156**: 354–361.

Rezaïki L, Lamberet G, Derré A, Gruss A & Gaudu P (2008) *Lactococcus lactis* produces short-chain quinones that cross-feed Group B *Streptococcus* to activate respiration growth. *Mol Microbiol* **67**: 947–957.

Richards VP, Lang P, Bitar PD, Lefébure T, Schukken YH, Zadoks RN & Stanhope MJ (2011) Comparative genomics and the role of lateral gene transfer in the evolution of bovine adapted *Streptococcus agalactiae*. *Infect Genet Evol* **11**: 1263–1275.

Ritchey TW & Seeley HW (1974) Cytochromes in *Streptococcus faecalis* var. *zymogenes* Grown in a Haematin-containing Medium. *J Gen Microbiol* **85**: 220–228.

Rosengren L & Winblad B (1976) Proteolytic activity of *Streptococcus mutans* (GS-5). *Oral Surg Oral Med Oral Pathol* **42**: 801–809.

Russell RR, Aduse-Opoku J, Sutcliffe IC, Tao L & Ferretti JJ (1992) A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. *J Biol Chem* **267**: 4631–4637.

Saier MH (1989) Involvement of the bacterial phosphotransferase system in diverse mechanisms of transcriptional regulation. *Res Microbiol* **140**: 349–352.

Saier MH, Chauvaux SJ, Cook GM, Deutscher J, Paulsen IT, Reizer J & Ye J-J (1996) Catabolite repression and inducer control in Gram-positive bacteria. *Microbiology* **142**: 217–230.

Salama M, Sandine W & Giovannoni S (1991) Development and application of oligonucleotide probes for identification of *Lactococcus lactis* subsp. *cremoris*. *Appl Environ Microbiol* **57**: 1313–1318.

Sanders JW, Leenhouts KJ, Haandrikman AJ, Venema G & Kok J (1995) Stress response in *Lactococcus lactis*: cloning, expression analysis, and mutation of the lactococcal superoxide dismutase gene. *J Bacteriol* **177**: 5254–5260.

Schachtele CF & Mayo JA (1973) Phosphoenolpyruvate-dependent glucose transport in oral streptococci. *J Dent Sci* **52**: 1209–1215.

Schleifer KH (1987) Recent changes in the taxonomy of lactic acid bacteria. *FEMS Microbiol Lett* **46**: 201–203.

Schleifer K, Kraus J, Dvorak C, Kilpper-Baelz R, Collins M & Fischer W (1985) Transfer of *Streptococcus lactis* and related streptococci to the genus *Lactococcus* gen. nov. *Syst Appl Microbiol* **6**: 183–195.

Schumacher MA, Seidel G, Hillen W & Brennan RG (2007) Structural mechanism for the fine-tuning of CcpA function by the small molecular effectors glucose 6-phosphate and fructose 1,6-bisphosphate. *J Mol Biol* **368**: 1042–1050.

Seidel G, Diel M, Fuchsbauer N & Hillen W (2005) Quantitative interdependence of coeffectors, CcpA and *cre* in carbon catabolite regulation of *Bacillus subtilis*. *FEBS J* **272**: 2566–2577.

Seki M, Iida K-I, Saito M, Nakayama H & Yoshida S-I (2004) Hydrogen peroxide production in *Streptococcus pyogenes*: involvement of lactate oxidase and coupling with aerobic utilization of lactate. *J Bacteriol* **186**: 2046–2051.

Sheng J & Marquis RE (2007) Malolactic fermentation by *Streptococcus mutans*. *FEMS Microbiol Lett* **272**: 196–201.

Siezen RJ, Renckens B, van Swam I, Peters S, van Kranenburg R, Kleerebezem M & de Vos WM (2005) Complete sequences of four plasmids of *Lactococcus lactis* subsp. *cremoris* SK11 reveal extensive adaptation to the dairy environment. *Appl Environ Microbiol* **71**: 8371–8382.

Siezen RJ, Starrenburg MJC, Boekhorst J, Renckens B, Molenaar D & van Hylckama Vlieg JET (2008) Genome-scale genotype-phenotype matching of two *Lactococcus lactis* isolates from plants identifies mechanisms of adaptation to the plant niche. *Appl Environ Microbiol* **74**: 424–436.

Siezen RJ, Bayjanov J, Renckens B, Wels M, van Hijum SAFT, Molenaar D & van Hylckama Vlieg JET (2010) Complete genome sequence of *Lactococcus lactis* subsp. *lactis* KF147, a plant-associated lactic acid bacterium. *J Bacteriol* **192**: 2649–2650.

Siezen RJ, Bayjanov JR, Felis GE, van der Sijde MR, Starrenburg M, Molenaar D, Wels M, van Hijum SA & van Hylckama Vlieg JE (2011) Genome-scale diversity and niche adaptation analysis of *Lactococcus lactis* by comparitive genome hybridization using multi-strain assays. *Microb Biotechnol* **4**: 383–402.

Sijpesteijn A (1970) Induction of cytochrome formation and stimulation of oxidative dissimilation by hemin in *Streptococcus lactis* and *Leuconostoc mesenteroides*. *Antonie Van Leeuwenhoek* **36**: 335–348.

Simon JP, Wargnies B & Stalon V (1982) Control of enzyme synthesis in the arginine deiminase pathway of *Streptococcus faecalis*. *J Bacteriol* **150**: 1085–1090.

Slee AM & Tanzer JM (1979) Phosphoenolpyruvate-dependent sucrose phosphotransferase activity in five serotypes of *Streptococcus mutans*. *Infect Immun* **26**: 783–786.

Snoep JL, de Graef MR, Teixeira de Mattos MJ & Neijssel OM (1992) Pyruvate catabolism during transient state conditions in chemostat cultures of *Enterococcus faecalis* NCTC 775: importance of internal pyruvate concentrations and NADH/NAD+ ratios. *J Gen Microbiol* **138**: 2015–2020.

Solem C, Koebmann BJ & Jensen PR (2003) Glyceraldehyde-3-phosphate dehydrogenase has no control over glycolytic flux in *Lactococcus lactis* MG1363. *J Bacteriol* **185**: 1564–1571.

Spellerberg B, Cundell DR, Sandros J, Pearce BJ, Idanpaan-Heikkila I, Rosenow C & Masure HR (1996) Pyruvate oxidase, as a determinant of virulence in *Streptococcus pneumoniae*. *Mol Microbiol* **19**: 803–813.

St Martin EJ & Wittenberger CL (1979) Characterization of a phosphoenolpyruvate-dependent sucrose phosphotransferase system in *Streptococcus mutans*. *Infect Immun* **24**: 865–868.

Suzuki T, Tagami J & Hanada N (2000) Role of F_1Fo-ATPase in the growth of *Streptococcus mutans* GS5. *J Appl Microbiol* **88**: 555–562.

Syu M-J (2001) Biological production of 2,3-butanediol. *Appl Microbiol Biotechnol* **55**: 10–18.

Tachon S, Brandsma JB & Yvon M (2010) NoxE NADH oxidase and the electron transport chain are responsible for the ability of *Lactococcus lactis* to decrease the redox potential of milk. *Appl Environ Microbiol* **76**: 1311–1319.

Takahashi S, Abbe K & Yamada T (1982) Purification of pyruvate formate-lyase from *Streptococcus mutans* and its regulatory properties. *J Bacteriol* **149**: 1034–1040.

Takahashi-Abbe S, Abe K & Takahashi N (2003) Biochemical and functional properties of a pyruvate formate-lyase (PFL)-activating system in *Streptococcus mutans*. *Oral Microbiol Immunol* **18**: 293–297.

Taniai H, Iida K-I, Seki M, Saito M, Shiota S, Nakayama H & Yoshida S-I (2008) Concerted action of lactate oxidase and pyruvate oxidase in aerobic growth of *Streptococcus pneumoniae*: role of lactate as an energy source. *J Bacteriol* **190**: 3572–3579.

Tanzer JM, Chassy BM & Krichevsky MI (1972) Sucrose metabolism by *Streptococcus mutans*, SL-I. *Biochim Biophys Acta* **261**: 379–387.

ten Brink B & Konings WN (1982) Electrochemical proton gradient and lactate concentration gradient in *Streptococcus cremoris* cells grown in batch culture. *J Bacteriol* **152**: 682–686.

ten Brink B, Otto R, Hansen UP & Konings WN (1985) Energy recycling by lactate efflux in growing and nongrowing cells of *Streptococcus cremoris*. *J Bacteriol* **162**: 383–390.

Tettelin H, Saunders NJ, Heidelberg J *et al.* (2000) Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. *Science* **287**: 1809–1815.

Tettelin H, Nelson KE, Paulsen IT *et al.* (2001) Complete genome sequence of a virulent isolate of *Streptococcus pneumoniae*. *Science* **293**: 498–506.

Teuber M (1995) The genus *Lactococcus*. *Genera of Lactic Acid Bacteria* (Holzapfel WH & Wood BJB, eds), pp. 173–234. Blackie Academic & Professional, London, UK.

Thomas TD, Turner KW & Crow VL (1980) Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris*: pathways, products, and regulation. *J Bacteriol* **144**: 672–682.

Thompson J (1980) Galactose transport systems in *Streptococcus lactis*. *J Bacteriol* **144**: 683–691.

Thompson J & Saier MHJ (1981) Regulation of methyl-β-d-thiogalactopyranoside-6-phosphate accumulation in *Streptococcus lactis* by exclusion and expulsion mechanisms. *J Bacteriol* **146**: 885–894.

Titgemeyer F & Hillen W (2002) Global control of sugar metabolism: a Gram-positive solution. *Antonie Van Leeuwenhoek* **82**: 59–71.

Tynkkynen S, Buist G, Kunji E, Kok J, Poolman B, Venema G & Haandrikman A (1993) Genetic and biochemical characterization of the oligopeptide transport system of *Lactococcus lactis*. *J Bacteriol* **175**: 7523–7532.

Udaka S, Koukol J & Vennesland B (1959) Lactic oxidase of pneumococcus. *J Bacteriol* **78**: 714–725.

Vadeboncoeur C (1984) Structure and properties of the phosphoenolpyruvate: glucose phosphotransferase system of oral streptococci. *Can J Microbiol* **30**: 495–502.

van Niel EWJ & Hahn-Hägerdal B (1999) Nutrient requirements of lactococci in defined growth media. *Appl Microbiol Biotechnol* **52**: 617–627.

van Rooijen RJ, van Schalkwijk S & de Vos WM (1991) Molecular cloning, characterization, and nucleotide sequence of the tagatose 6-phosphate pathway gene cluster of the lactose operon of *Lactococcus lactis*. *J Biol Chem* **266**: 7176–7181.

Vandamme P, Pot B, Gillis M, de Vos P, Kersters K & Swings J (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiol Rev* **60**: 407–438.

Viana R, Monedero V, Dossonnet V, Vadeboncoeur C, Pérez-Martínez G & Deutscher J (2000) Enzyme I and HPr from *Lactobacillus casei*: their role in sugar transport, carbon catabolite repression and inducer exclusion. *Mol Microbiol* **36**: 570–584.

Wegmann U, O'Connell-Motherway M, Zomer A *et al.* (2007) Complete genome sequence of the prototype lactic acid bacterium *Lactococcus lactis* subsp. *cremoris* MG1363. *J Bacteriol* **189**: 3256–3270.

Whittenbury R (1964) Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. *J Gen Microbiol* **35**: 13–26.

Williams AM, Fryer JL & Collins MD (1990) *Lactococcus piscium* sp. *nov*. a new *Lactococcus* species from salmonid fish. *FEMS Microbiol Lett* **68**: 109–113.

Woese CR (1987) Bacterial evolution. *Microbiol Mol Biol Rev* **51**: 221–271.

Wood BJB & Warner PJ (2003) *Genetics of Lactic Acid Bacteria*. Kluwer Academic/Plenum Publishers, New York.

Würsch P & Koellreutter B (1985) Maltotriitol inhibition of maltose metabolism in *Streptococcus mutans* via maltose transport, amylomaltase and phospho-alpha-glucosidase activities. *Caries Res* **19**: 439–449.

Wydau S, Dervyn R, Anba J, Dusko ES & Maguin E (2006) Conservation of key elements of natural competence in *Lactococcus lactis* ssp. *FEMS Microbiol Lett* **257**: 32–42.

Yamamoto Y, Higuchi M, Poole LB & Kamio Y (2000) Role of the *dpr* product in oxygen tolerance in *Streptococcus mutans*. *J Bacteriol* **182**: 3740–3747.

Yamamoto Y, Poyart C, Trieu-Cuot P, Lamberet G, Gruss A & Gaudu P (2005) Respiration metabolism of Group B *Streptococcus* is activated by environmental haem and quinone and contributes to virulence. *Mol Microbiol* **56**: 525–534.

Zhao Y, Liu G, Li S, Wang M, Song J, Wang J, Tang J, Li M & Hu F (2011) Role of a type IV–like secretion system of *Streptococcus suis* 2 in the development of streptococcal toxic shock syndrome. *J Infect Dis* **204**: 274–281.

Zomer AL, Buist G, Larsen R, Kok J & Kuipers OP (2007) Time-resolved determination of the CcpA regulon of *Lactococcus lactis* subsp. *cremoris* MG1363. *J Bacteriol* **189**: 1366–1381.